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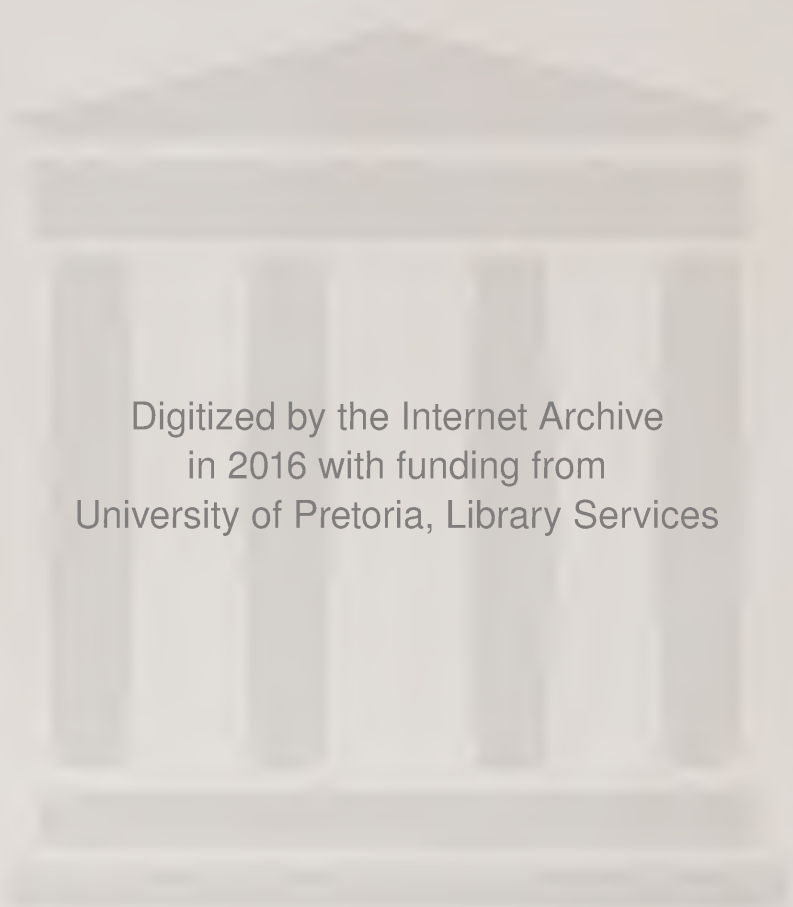
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DEPARTMENT OF
AGRICULTURE.

EIGHTEENTH REPORT
OF THE
DIRECTOR OF VETERINARY SERVICES
AND ANIMAL INDUSTRY,
ONDERSTEEPOORT,
PRETORIA.
AUGUST, 1932.
PART II.

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ONDERSTEEPOORT LABORATORIES,
PRETORIA, SOUTH AFRICA,
August, 1932.

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Onderstepoort Laboratories.**

- Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1903-4.*
Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1904-5.*
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First Report of the Director of Veterinary Research, August, 1911.*
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Fifth and Sixth Reports of the Director of Veterinary Research, April, 1918.*
Seventh and Eighth Reports of the Director of Veterinary Research, April, 1918.*
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P. J. DU TOIT,
Director of Veterinary Services and Animal Industry.

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WALD AND G.
DE KOCK.



A Study of the Mineral Content and Feeding Value of Natural Pastures in the Union of South Africa—(First Report).

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V. SUMMARY.

I. INTRODUCTION.

PASTURE studies may be regarded as one of the most important branches of modern agricultural research. It is of recent origin and has already yielded far-reaching results. Outstanding is the work of Woodman and his collaborators at Cambridge on the effect of growth on the feeding value of pasture. The economic value of this work, which is closely associated with what is known as the new method of grassland management, can hardly be overestimated. Pasture, herbage, if correctly managed and timely used may be regarded as a protein concentrate, whereas the same pasture when allowed to mature may have low feeding value and show a wide nutritive ratio. It is admitted, of course, that the application of the findings of Woodman and his co-workers hold good primarily for the climatic conditions under which they worked, which are, generally speaking, a high rainfall, well distributed over the year, and a temperate climate. But it is fully realized that pasture research under other conditions may have equally important bearings on and suggest vast changes in the existing methods of pasture management. It is not too early to say that the preliminary work done by Staples and Taylor (1926-1931) at the Cedara School of Agriculture, Natal, is already indicative of many errors in the system of pasture management at present in vogue in South Africa.

Mention should also be made here of the work of Orr and his staff at the Rowett Research Institute, on the feeding value of pasture from the aspect of its mineral content, and of the investigations of Stapledon and his colleagues (1924-1931) at the Welsh Plant Breeding Station into chemical composition and seasonal variations.

In the Union of South Africa the need for improvement of pastures is much more urgent than in England and other European countries. Approximately 95 per cent. of our country consists of natural pasture and most of this is of very low carrying capacity. This need was realized by some of the early investigators and Hutcheon on the animal husbandry side and Juritz on the chemical side, carried out some very valuable work twenty to thirty years ago.

Amongst the later investigators who stressed the need for supplementing the deficiencies of our pastures, pride of place must be given to Theiler. He and his co-workers began their investigations with the study of lambsiekte, a disease which was found to have its ultimate cause in the phosphorus deficiency of the soil and pasture. They pursued their researches into this deficiency further and found that it played a pre-eminent rôle in the nutrition of animals. By supplying the deficient minerals they were able to breed prime animals on veld which ordinarily only produced scrubs. In these investigations the study of the pasture itself occupied a prominent place and a large volume of data has been collected at Onderstepoort mainly on the chemical composition of grasses.

As this work progressed it was found that phosphorus deficiency was far commoner in the Union than was thought to be the case at first. But the mere knowledge that the grasses from a certain area were deficient in phosphorus was not sufficient. In order to obtain useful information on their feeding value a systematic study became necessary of such factors as soil composition, climatic conditions, seasonal variation, stage of development of the plant, differences due to species, etc.

It is our intention to embody the study of most of these aspects of the problem in the present investigation. Nevertheless the work must remain incomplete because of the immense area to be covered and the limitation of staff and funds. That we were able to undertake the present investigations at all on the lines contemplated, is due in large measure to the generosity of the Empire Marketing Board, who supplied part of the funds and to whom grateful acknowledgment is made herewith.

II. OUTLINE OF THE INVESTIGATION.

1. ORIGIN OF THE SCHEME.

The findings of recent investigators that a phosphorus deficiency is widespread in many parts of Africa, Australia and other countries, emphasized the scope of this problem and the necessity of studying it further. The possibility of other mineral deficiencies playing an important rôle was also kept in mind when the scheme was under consideration.

At the same time it was realized that excellent opportunities would become available during the course of the investigation for studying the feeding value of pastures as far as the figures for crude protein, fibre, carbohydrates plus fat content could provide a

criterion. In other words, the need of more detailed data on pastures generally and their bearing on problems of nutrition and disease was felt when this investigation was conceived.

2. OBJECT OF THE INVESTIGATION.

The investigation was suggested by the need of more accurate knowledge in South Africa regarding the distribution of phosphorus-deficient and phosphorus-sufficient areas. The first object of the survey was, therefore, to collect data which would enable us to map out the Union of South Africa into areas according to the phosphorus content of the soil and pasture.

But the investigation was not intended to have a local application only. As indicated above, the problem applies to other parts of the Empire (East and West Africa, Australia, etc.) and other countries as well, and it was our desire to test out various methods of research in South Africa which would afterwards be of benefit to those other countries. These methods are discussed here briefly.

3. METHODS EMPLOYED.

In previous work in South Africa phosphorus deficiency in an area was always determined by the analysis either of the soil itself or of pasture grasses grown in that area. In 1927, Malan, Green and Du Toit showed that phosphorus deficiency is also reflected in the blood of animals grazing on such pasture. In the present investigation it was decided, therefore, to analyse—

- (a) the soil from the selected areas,
- (b) grasses growing on that soil,
- (c) blood of cattle grazing on such pasture.

It was hoped to establish a definite correlation between these three sets of data and possibly to determine whether any one of these procedures yielded sufficiently accurate results to render the other two superfluous. The advantages and disadvantages of these methods will be discussed below.

4. SCOPE OF THE ANALYTICAL WORK AND METHODS OF ANALYSIS.

Not only phosphorus but also Ca, Mg, Na, K and Cl were included; while analyses for crude protein, fibre and carbohydrate plus fat have also been carried out.

The methods employed for grass and blood analyses for inorganic constituents are those described by Malan and Van der Lingen (1931). The method for sodium has been modified slightly in that it was found unnecessary to remove phosphorus by the addition of alcoholic zinc acetate. After the precipitation of sodium the precipitate is washed with a saturated solution of uranyl zinc sodium acetate in 96 per cent. alcohol and stirred with a small glass rod. This procedure is necessary to break up the uranyl phosphate which forms as the top layer of the precipitate in the centrifuge tube.

Protein and fibre were determined according to the methods described by Wood (1911). Ether soluble extracts were not determined as their values show little variation, and are insignificantly small compared with those for carbohydrates, while the method takes

up a great deal of time when large numbers of determinations have to be made. The results, therefore, yield crude protein, fibre, carbohydrates plus ether soluble extract, silica, and the individual inorganic constituents mentioned above. Only the available inorganic constituents were determined in the soil by shaking 50 grams of soil with 250 c.c. N/20 hydrochloric acid solution for 60 minutes, filtering and washing to 1,000 c.c. This extract was used for analysis and the micro-methods, described by Steenkamp (1931), employed. The extract for chlorine was made by substituting N/500 H_2SO_4 for HCl and following the same procedure.

5. TECHNIQUE.

The technique offers no difficulty after the usual experience with micro-chemical work has been gained. The results of inorganic phosphorus in the blood are affected by the interval between the time of bleeding in the field and the actual analyses in the laboratory, a matter of about 3 days usually. Hydrolysis of organic phosphorus results in an increase of inorganic phosphorus as explained by Malan (1930). Hence the procedure followed is such that would expedite the despatch of the blood from the field to Onderstepoort. Briefly, the blood is drawn, precipitated and immediately despatched to Onderstepoort, where it is analysed for inorganic phosphorus on arrival. Under those conditions the hydrolysis, although by no means negligible, does not increase low values sufficiently to be classed with figures indicating phosphorus sufficiency, although, obviously, borderline cases are likely to be missed. In any case, the object of the investigation is not to obtain correct values for inorganic phosphorus in the blood of animals but to diagnose phosphorus deficiency or sufficiency, and for that purpose the increased values provide enough data.

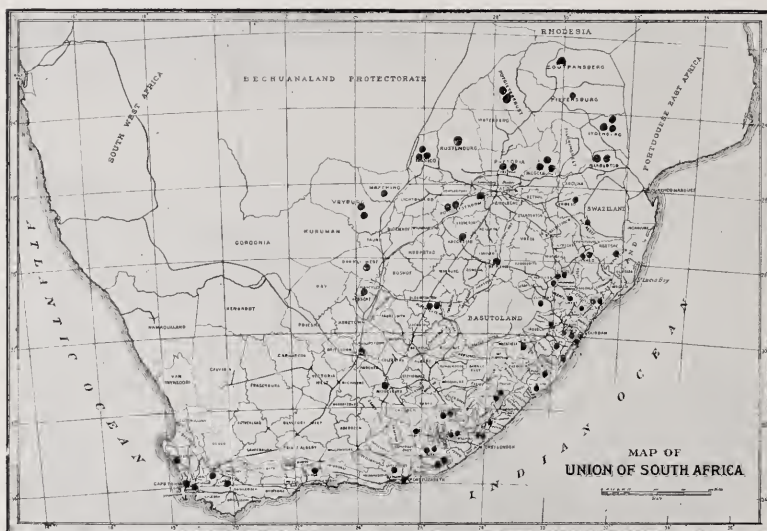
There is an upward grading of all values after hydrolysis, which means that low values for inorganic phosphorus are higher and that normal values will lie above that level, but the figures still lend themselves to interpretation, provided the age and class of bovine (lactating or dry cow, ox, etc.) and the time between bleeding and analysis be given. At present data are being collected on the monthly inorganic phosphorus content of the blood of bovines of different ages under phosphorus deficient and phosphorus sufficient conditions in the Vryburg area. The inorganic phosphorus is determined at bleeding time, while separate portions of the blood of each animal are treated in the regular way for all the samples of the surveys, forwarded to Onderstepoort and analysed here after different periods of hydrolysis. Correction values may then be calculated for both low and high figures under the conditions that the surveys are actually carried out. This point will again be referred to later in the publication.

6. EXPERIMENTAL PLAN.

(a) *Mineral Survey of the Union.*

One part of the scheme deals with a survey of the Union. Samples of soil, vegetation and blood are sent in by about forty Government field Veterinary Officers at the four seasons of the year. Each officer selects at least one area for collection in his district and makes successive collections from it provisionally for two years, in

order to study the effect of climate on the composition of the soil, vegetation and the effect of the latter on the blood constituents. On the accompanying map the areas from which samples have been collected so far are marked.



EXPERIMENTAL PLAN: MINERAL SURVEY OF THE UNION.

★ Shows areas from which samples have been collected.

The first survey took place in May, 1930, the second in May, 1931, after which arrangements were made for the regular carrying out of surveys annually in October, January, April and July.

Soil samples are taken in the ordinary way from a paddock, while collections of each of the prominent grasses in the paddock are made. Blood is drawn from ten bovines—preferably dry cows, which receive no supplementary feeding. Briefly the procedure is as follows: About a month before a survey is to take place a circular letter is forwarded to all Government field Veterinary Officers stating exactly what is to be done and notifying the officers that small bottles containing trichloroacetic acid for the collection and precipitation of blood will be despatched in due course. A copy of such a circular is printed below:—

P.O. Onderstepoort,
Pretoria (Date.)

To all Senior and Government Veterinary Officers.

MINERAL SURVEY No. 6.

Time: The next survey will take place from 1st July to 31st July, 1932, and I trust that all officers without exception will provide the material during that time.

Farm:

Collections of soil, pasture, and blood samples are to be made.

(i) *Soil Samples.*—In collecting soil samples officers must use their own discretion. For instance, in a region where grass is abundant the bulk of the grass roots will probably not penetrate to a greater depth than about 12 inches,

and a sample of soil taken to that depth will probably be a good representative sample; on the other hand, for shrubs, it may be necessary to take samples down to about 2 feet or more. Preliminary work such as digging up shrubs or grass in order to find out the depth of the roots is essential, and the actual samples can be taken according to the findings of the preliminary work.

After selection of the farm or portion of it, where soil samples have to be taken, a patch of about 12 by 12 inches is cleared of all vegetation. A hole of the required depth is dug next to the clear patch, and a slice about 2 inches wide cut down the perpendicular side, where the vegetation has been removed. This is transferred to the bag provided. The operation is repeated in at least four places, so as to obtain a representative soil sample of the farm or portion of it decided upon; all four samples are transferred to the same bag. If the soil is very moist it should be air-dried before despatch to Onderstepoort.

(ii) *Grass Samples*.—Should an officer decide, after consultation with the farmer, that there are half a dozen varieties of edible grasses in the area under survey, he should collect at least one pound of each variety *separately*, thereby assisting the process of classification which has to be carried out at Onderstepoort. The grass must be cut as near the ground as possible and made air-dry. Each variety is wrapped in paper and all are then placed in a bag or sugar-pocket and forwarded to Onderstepoort by rail.

(iii) *Blood Samples*.—Each officer will be provided with two sets of bottles before the beginning of July. The ten small bottles contain sodium citrate as anticoagulant and are to be used for the collection of blood. The larger bottles contain 100 c.c. of 2·5 per cent. trichloroacetic acid solution for the precipitation of the blood proteins. After bleeding into the small bottles, 25 c.c. blood are transferred in a pipette to the trichloroacetic acid solution. The bottle is then corked and shaken vigorously for about half a minute, when it is ready for dispatch to Onderstepoort. It is not necessary to clean the pipette after the transfer of every sample of blood to the trichloroacetic acid solution, unless rinsing is obviously necessary, when only distilled water should be used. Bovine blood is preferred, and only from animals which do not receive supplementary feeding in any form, least of all any phosphatic product, but which are entirely dependent upon the pasture for their existence. Owners may feel reluctant to have their poorest animals bled, and even you may be inclined to select the best conditioned animals. This clearly partly defeats our purpose, as a figure obtained for inorganic phosphorus in the blood of the best conditioned animals will not be a good criterion of the phosphorus sufficiency or deficiency of the veld unless some of the poorest animals or, alternately, 5 poor ones and 5 that are in good condition be selected. Full-grown dry cows are the best types to select, for, whereas in lactating cows milk production causes an extra drain of phosphorus, oxen usually produce work, and the phosphorus reserve of young stock is undoubtedly high, causing the blood phosphorus to remain high for a considerable time in spite of deficiency. Full-grown animals must, therefore, be selected wherever possible and in the following order of merit:—

1. Full-grown dry cows, pregnant or not.
2. Full-grown oxen with a note about the work done.
3. Full-grown lactating cows with a note about milk yield.
4. Younger animals older than 18 months.

N.B.—Please give all the available details about individual animals bled, e.g. age, sex, etc., on the form provided. The blood is to be forwarded to Onderstepoort by post *immediately after precipitation*.

General.—(a) Kindly fill in the form placed in the wooden box containing the bottles and dispatch it with the precipitated blood samples.

(b) All empty bleeding bottles should be returned by placing them in the bags containing the grass samples.

(c) All the samples, i.e. soil, grass, and blood, are to be taken on the same day or on successive days.

(d) Animals must not be driven any distance before bleeding, as the inorganic phosphorus of the blood rises as a result of exercise.

(Sgd.) DIRECTOR OF VETERINARY SERVICES.

The form referred to in (a) of the last paragraph is printed below:—

Name of Veterinary Officer.....
 Farm and district from which samples were collected.....
 Name and address of owner.....
 Date when bled.....
 Date of dispatch of samples.....
 Blood samples: 1.....
 2.....
 3.....
 4.....
 5.....
 6.....
 7.....
 8.....
 9.....
 10.....

Under blood samples insert whether from heifer, dry cows, lactating cow, ox, etc., stating approximate age and giving details about condition, health, and breed.

Rainfall: January.....
 February.....
 March.....
 April.....
 May.....
 June.....
 July.....
 August.....
 September.....
 October.....
 November.....
 December.....

The samples of vegetation are identified on arrival and selected specimens placed in the herbarium for future reference, if necessary. Each variety is labelled green, mature or mixed, according to its appearance, then finely ground in a mill, after which it is ready for analysis. About 200 grass samples are received for each survey, which means that about 1,600 analyses have to be made before the following survey begins.

It is evident that the above scheme, although elaborate, is incomplete. An estimate—admittedly very important—may be formed of the feeding value of grasses at the different seasons, but such factors as the variation of the mineral content of separate species with age, will remain unknown, and hence direct comparisons of the results of the analyses of different species of grasses from the same area or of the same species from different areas must remain unwise when different soil and climatic conditions of which very little is known, exist. Besides, the period of growth when one is dealing with both early and late growing varieties must be known in order to make such comparisons. In order to study these factors further the survey was extended to include experimental plots.

(b) Experimental Plots.

This phase of the work began in 1931, when important indigenous grasses were planted in separate plots of virgin soil, tended until good growth was assured, after which only weeding took place. At present about 20 such plots are in full growth and constant additions are being made. Older plots are in existence in Pretoria about 7 miles from Onderstepoort on a different kind of soil for a study of the influence of soil type on the composition of grasses both by the Division of Chemistry and by the writers.

The objects of the plot experiments may be briefly summarized as follows:

- (1) The effect of growth on the chemical composition of pasture. For this purpose monthly, two-monthly, three-monthly, four-monthly, etc., cuttings are analysed from each plot, i.e. of each variety of grass.
- (2) The variation in chemical composition of different species of grasses of the same period of growth.
- (3) The effect of seasonal variation in growth on the chemical composition and on the yield of grass.
- (4) The chemical composition of successive cuttings of the same variety of grass. This means that, if a portion of a plot is cut for a sample of monthly growth, the same portion will be cut again after another month, i.e. when a two-monthly sample is taken from another portion of the plot; this procedure is repeated for both one-monthly and two-monthly cuttings, etc., at later samplings.
- (5) The chemical composition of different parts of the same plant at different stages of growth. For this purpose, leaves, stalks and haulms will be analysed separately at the different periods.

Climatic conditions will obviously play a most important part in the work on plot experiments, but these will be recorded and considered along with the data.

(c) Extension of Blood Analyses.

A recent development of the experimental plan is towards a greater utilization of the results of blood analysis for the delimitation of phosphorus-deficient and phosphorus-sufficient areas of the Union. The advantage obviously lies in the fact that blood can be drawn with great ease by the Government Veterinary Officers from animals in a number of areas in their districts, forwarded to Onderstepoort at very little cost and analysed for inorganic phosphorus with a minimum of labour. This application of blood analysis is based upon the findings of Malan, Green and Du Toit (1927), viz., that animals suffering from aphosphorosis show a low figure for inorganic phosphorus in their blood.

III. A FEW PROVISIONAL RESULTS.**1. THE CHEMICAL COMPOSITION OF SOILS* AND PASTURE† FROM DIFFERENT AREAS IN THE UNION.**

* The soil analyses were kindly undertaken by the Division of Chemistry, Pretoria.

† Mr. G. W. B. van der Lingen, M.Sc., Chemist, was responsible for the analytical work of the Mineral Survey in May, 1930.

TABLE 11.

VALUES IN GM. PER 100 GM. DRY MATERIAL.

N for pasture is given as crude protein. The numerals in column one refer to the surveys in May, 1930, May, 1931, and October, 1931, respectively.

District and Number of Survey.	P ₂ O ₅ .	CaO.	MgO.	K ₂ O.	Na ₂ O.	Cl.	N.	Fibre.	Carb. + Ether Sol. Ext.	Farm.	Nature of Pasture.
1. Middelburg, Transvaal	Soil... 1	.442	.07	.031	.0098	.0017	.099	—	—	Rooipoort No. 152	—
	2	.081	Nil	.025	.0075	.035	.074	—	—	Aberdeen No. 291..	—
	3	.048	Trace	.021	.0043	Nil	.007	—	—	Rooipoort No. 8..	—
	Pasture 1	.55	.22	.94	.12	.12	4.2	—	—	Rooipoort No. 152	Mixed.
	2	.25	.13	1.02	.05	.24	3.2	—	—	Aberdeen No. 291..	Mixed.
	3	.34	.14	.73	.01	.19	5.7	35.4	56.3	Rooipoort No. 8..	Mixed.
Krugersdorp, Transvaal	Soil... 1	.137	.083	.0259	.0090	.0017	.088	—	—	Hartebeestfontein No. 51	—
	2	—	—	—	.0093	Nil	—	—	—	Hartebeestfontein No. 51	—
	3	.118	.066	.0265	.0093	Nil	.132	—	—	" "	—
	Pasture 1	.46	.32	.63	.28	.28	4.2	—	—	" "	Mixed, mainly mature.
	2	—	—	—	—	—	—	—	—	Hartebeestfontein No. 51	Mixed, practically all mature.
	3	.36	.11	.34	.01	.05	3.4	40.5	54.9	" "	—
3. Barberton, Transvaal	Soil... 1	.399	.180	.0268	.0146	Nil	.078	—	—	Brooklyn.....	—
	2	.23	.129	.0475	.0087	.0035	.111	—	—	Brooklyn.....	—
	3	.41	.45	1.04	.36	.28	5.7	—	—	" "	Mixed, mainly mature.
	Pasture 1	.34	.18	.59	.03	.14	3.8	40.3	53.5	Brooklyn.....	Old dry mature.
	2	—	—	—	.0215	Nil	.124	—	—	Frischgewaagd No. 82	—
	3	.078	.071	.0332	.0164	.0052	.0560	—	—	De Grootboom No. 214	—
4. Lydenburg, Transvaal	Soil... 1	.171	.207	.0412	.0038	Nil	.074	—	—	" "	—
	2	.65	.407	.124	.0164	.0052	.0560	—	—	Frischgewaagd No. 82	Mixed, mainly mature.
	3	.0115	.0043	.17	.038	.24	3.9	—	—	De Grootboom No. 214	Mixed, practically all mature.
	Pasture 1	.44	.38	.24	.17	.20	6.1	—	—	" "	Old dry mature.
	2	.49	.17	1.52	.54	.64	2.7	—	—	" "	—
	3	.25	.20	.47	.01	.08	2.7	37.6	58.2	" "	—

TABLE 1—(continued).

District and Number of Survey.		P ₂ O ₅ .	CaO.	MgO.	K ₂ O.	Na ₂ O.	Cl.	N.	Fibre.	Carb. + Ether Sol. Ext.	Farm.	Nature of Pasture.
5. Marico Transvaal	Soil... 1	·0004	·106	·145	·0195	·0140	·0035	·043	—	—	Nietverdiend No. 196	—
	2	·00058	·038	Nil	·0082	·0073	·0052	·0378	—	—	Kaaplaats No. 97...	—
	3	·00048	·022	Nil	·0123	·0023	Nil	·069	—	—	" "	Mixed, mainly mature.
	Pasture 1	·23	·38	·36	1·12	·13	·26	4·6	—	—	Nietverdiend No. 196	Mixed, practically all mature.
	2	·35	·35	·15	·51	·07	·15	2·5	—	—	Kaaplaats No. 97...	Old dry mature.
	3	·09	·24	·12	·18	·03	·66	2·1	44·4	52·3	" "	" "
6. Zoutpansberg, Transvaal	Soil... 1	—	·399	·086	·0481	·0149	·0035	·0434	—	—	Govt. Ranching Stn.	—
	2	·0431	1·747	·160	·0492	·0052	·0017	·035	—	—	" "	—
	3	·4918	—	—	·119	·12	·39	4·3	—	—	" "	Mixed, practically all mature.
	Pasture 1	·16	·47	·18	·19	·16	·22	3·5	41·3	53·1	Govt. Ranching Stn.	Mixed, mainly mature.
	2	·44	·38	·22	·63	·16	·39	—	—	—	" "	—
	3	—	—	—	—	—	—	—	—	—	" "	—
7. Potgietersrust, Transvaal	Soil... 1	·00035	·140	·133	·0263	·0185	·0017	·059	—	—	Hebron No. 715....	—
	2	·0041	1·205	·407	·0408	·0084	·0017	·0952	—	—	Mooiegelegen No. 635	—
	3	—	—	—	—	—	—	—	—	—	" "	—
	Pasture 1	·14	·34	·25	·60	·14	·18	3·5	—	—	Hebron No. 715....	Mixed.
	2	·28	·39	·21	1·56	·10	·45	4·1	—	—	Mooiegelegen No. 635	Mainly mature.
	3	—	—	—	—	—	—	—	—	—	" "	—
8. Piet Retief, Transvaal	Soil... 1	—	·074	Nil	·0167	·0125	·0052	·1442	—	—	Derby No. 56.....	—
	2	·00015	·114	·044	·0121	·0059	Nil	·189	—	—	" "	—
	3	·0019	—	—	—	—	—	—	—	—	" "	—
	Pasture 1	·09	·34	·10	·58	·07	·20	2·7	—	—	Derby No. 56.....	Mixed, mainly mature.
	2	·18	·39	·16	·47	·05	·16	3·0	36·9	58·1	" "	Old dry mature.
	3	—	—	—	—	—	—	—	—	—	" "	—
9. Pietersburg, Transvaal	Soil... 1	·00036	·140	·061	·0268	·0191	·0017	·070	—	—	Wildheestfontein	—
	2	·00059	·110	·074	·0339	·0113	Nil	·070	—	—	No. 89	—
	3	·00045	·097	·051	·0339	·0045	Nil	·069	—	—	" "	—
	Pasture 1	·16	·43	·23	·89	·10	·13	4·6	—	—	" "	Mixed, mainly mature.
	2	·18	·25	·13	·68	·04	·19	3·5	—	—	" "	" "
	3	·14	·34	·16	·42	·01	·11	2·8	31·0	59·5	" "	" "

TABLE 1—(continued).

District and Number of Survey.	P ₂ O ₅ .	CaO.	MgO.	K ₂ O.	Na ₂ O.	Cl.	N.	Fibre.	Carb. + Ether Sol. Ext.	Farm.	Nature of Pasture.
10. Portchefstroom, Transvaal	Soil... 1	.00037	.185	.086	.0278	.0017	.102	—	—	Rietfontein No. 503	—
	2	.00053	.181	.100	.0167	.014	.130	—	—	Mimosa Park.....	—
	3	.00030	.193	.138	.0039	NH	—	—	—	" "	—
Pasture 1	1	.16	.51	.19	.12	.13	3.3	—	—	Rietfontein No. 503	Mixed, practically all mature.
	2	.14	.38	.20	.09	.20	3.3	—	—	Mimosa Park.....	Mixed.
	3	.07	.22	.08	.01	.04	2.4	35.4	61.0	" "	Mixed, practically all mature.
11. Ernpelo, Transvaal	Soil... 1	.0061	.207	NH	.0172	NH	.0546	—	—	Leliefontein.....	—
	2	—	—	—	—	—	—	—	—	" "	—
	3	.14	.36	.20	.07	.13	3.7	—	—	Leliefontein.....	Mixed, mainly mature.
Pasture 1	1	.23	.34	.25	.11	.34	6.4	30.8	55.5	" "	Mixed, mainly green grass and shrubs.
12. Ixopo, Natal..	Soil... 1	.00053	.058	.079	.0098	NH	.3234	—	—	Stanton.....	—
	2	—	—	—	—	—	—	—	—	" "	—
	3	.11	.22	.18	.08	.21	2.8	—	—	Stanton.....	Mixed.
Pasture 1	1	.23	.27	.24	.07	.26	5.1	34.6	57.9	" "	"
13. Vryheid, Natal	Soil... 1	.00055	.035	.047	.0168	NH	.052	—	—	Uitvlugt.....	—
	2	.00053	.114	.066	.0274	.0132	.130	—	—	Bergendal.....	—
	3	.00024	.112	.095	.0154	NH	.125	—	—	" "	—
Pasture 1	1	.11	.43	.23	.65	.11	2.8	—	—	Uitvlugt.....	Mixed, mainly mature.
	2	.12	.27	.20	.70	.22	2.3	—	—	Bergendal.....	Mixed.
	3	.14	.34	.19	.47	.09	4.3	19.8	66.3	" "	"
14. Nongoma, Natal	Soil... 1	.00048	.055	.119	.011	.0035	.168	—	—	Nongoma Township	—
	2	.00044	.177	.097	.0111	.0017	.1010	—	—	Zulu Nat. Inst.....	—
	3	—	—	—	—	—	—	—	—	" "	—
Pasture 1	1	.13	.24	.18	.14	.17	3.9	—	—	Nongoma Township	Mixed, practically all mature.
	2	.18	.36	.35	.35	.53	4.8	—	—	Zulu Nat. Inst.....	Mixed.
	3	—	—	—	—	—	—	—	—	" "	—

TABLE 1—(continued).

District and Number of Survey.	P ₂ O ₅ .	CaO.	MgO.	K ₂ O.	Na ₂ O.	Cl.	N.	Fibre.	Carb. + Ether Sol. Ext.	Farm.	Nature of Pasture.
15. Pine Town, Natal	{ Soil... 1 2 3	{ .00055 2 3 0.00076	{ .113 -0.40 -0.49	{ .062 Nil -0.37	{ .029 -0.175 -0.296	{ .0074 -0.084 -0.048	{ .0017 -0.052 -0.105	{ .083 -0.518 -0.67	{ — — —	{ Marianhill... Zeekoegat... Marianhill...	{ — — —
	{ Pasture 1 2 3	{ .10 -0.21 -0.14 -0.22 -0.14	{ .21 -0.20 -0.08 -0.37 -0.09	{ .06 -0.22 -0.08 -0.37 -0.09	{ .06 -0.22 -0.10 -0.37 -0.49	{ .05 -0.20 -0.20 -0.16	{ 2.7 3.6 3.6 3.6	{ — — — 39.0*	{ — — — 52.9	{ Marianhill... Zeekoegat... Marianhill...	{ Mixed, practically all mature. Mixed. Mixed.
16. Eslowe, Natal.	{ Soil... 1 2 3	{ .00054 2 3 0.00067	{ .050 -0.37 -0.40	{ .064 Nil Nil	{ .0150 -0.124 -0.069	{ .0099 -0.094 -0.056	{ .0087 -0.207 -0.122	{ .272 -0.546 -0.59	{ — — —	{ Arcadia... Lots Nos. 45/48, En- tument "	{ — — —
	{ Pasture 1 2 3	{ .14 -0.39 -0.28 -0.29	{ .26 -0.13 -0.13 -0.13	{ .06 -0.26 -0.13 -0.13	{ .13 -0.70 -0.17 -0.57	{ .13 -0.17 -0.12	{ .20 -0.43 -0.37	{ 5.28 2.5 3.3	{ — — —	{ Arcadia... Lots Nos. 45/48, En- tument "	{ Mixed, mainly mature. " Mixed.
17. Greytown, Natal	{ Soil... 1 2 3	{ .00033 2 3 0.00044	{ .266 -0.246 -0.191	{ .049 -0.105 -0.081	{ .037 -0.442 -0.285	{ .019 -0.109 -0.088	{ .0017 -0.035 -0.032	{ .193 -0.366 -0.330	{ — — —	{ Area No. 24... " "	{ — — —
	{ Pasture 1 2 3	{ .17 -0.18 -0.36 -0.44	{ .48 -0.13 -0.73 -0.36	{ .14 -0.13 -0.19 -0.19	{ .78 -0.73 -1.15 -0.78	{ .02 -0.04 -0.09	{ .11 -0.19 -0.45	{ 4.1 2.5 10.0	{ — — —	{ " " "	{ Mixed. " Green.
18. Port Shepstone, Natal	{ Soil... 1 2 3	{ .0006 2 3 0.0011	{ .001 -0.76 -0.27	{ Nil -0.38 -0.11	{ .0105 -0.112 -0.06	{ .0077 -0.132 -0.14	{ .0087 Nil -0.37	{ .0476 -0.098 3.4	{ — — —	{ Melbourne... " "	{ — — —
	{ Pasture 1 2 3	{ .09 -0.11 -0.11	{ .11 -0.19 -0.19	{ .11 -0.19 -0.19	{ .76 -0.69 -0.69	{ .14 -0.26 -0.26	{ .37 -0.51 -0.51	{ 3.4 3.1 3.1	{ — — —	{ Melbourne... " "	{ Mixed, mainly mature. " "
19. Bartelevorth, Cape Province	{ Soil... 1 2 3	{ .00064 2 3 0.00099	{ .274 -0.330 -0.330	{ .131 -0.103 -0.103	{ .0235 -0.276 -0.276	{ .0282 -0.147 -0.147	{ .0035 -0.175 -0.175	{ .162 -0.231 -0.231	{ — — —	{ Woodlands... " "	{ — — —
	{ Pasture 1 2 3	{ .21 -0.15 -0.15	{ .45 -0.31 -0.31	{ .19 -0.07 -0.07	{ .03 -0.74 -0.74	{ .32 -0.16 -0.16	{ .25 -0.38 -0.38	{ 5.2 4.1 4.1	{ — — —	{ Woodlands... " "	{ Mixed, mainly mature. Mixed. Mixed.

TABLE 1—(continued).

		5									
District and Number of Survey.	P ₂ O ₅ .	CaO.	MgO.	K ₂ O.	Na ₂ O.	Cl.	N.	Fibre.	Carb. + Ether Sol. Ext.	Farm.	Nature of Pasture.
20. Kingwilliams- town, Cape Province	Soil... 1	-182	-078	-0294	-0174	NH	-144	—	—	Gleniffer.....	—
	2	-00090	-111	-0417	-0258	-0017	-2058	—	—	Rockdale.....	—
	3	-00004	-116	-0253	-0125	-0052	-088	—	—	".....	—
21. Kokstad, Cape Province	Pasture 1	-40	-16	-57	-10	-11	3-3	—	—	Gleniffer.....	Mixed, mainly mature.
	2	-09	-18	-64	-12	-21	1-9	—	—	Rockdale.....	Green.
	3	-36	-20	1-51	-26	-55	9-5	33-4	55-5	".....	"
22. Konanza, Cape Province	Soil... 1	-311	-145	-0362	-0277	NH	-126	—	—	Koppieskraal.....	—
	2	-00017	-351	-0433	-0174	-0017	-1708	—	—	".....	—
	3	-00038	-371	-0309	-0102	NH	-148	—	—	".....	—
23. Umtata, Cape Province	Pasture 1	-46	-25	-82	-14	-14	4-7	—	—	".....	Mixed, mainly mature.
	2	-23	-29	-20	-81	-15	3-6	—	—	".....	Mixed.
	3	-23	-30	-67	-09	-23	4-6	34-8	58-9	".....	"
24. Lushikisid, Cape Province	Soil... 1	-237	-074	-0276	-0162	NH	-165	—	—	Farm Haddon.....	Mature.
	2	-181	-084	-0271	-0149	-0035	-1386	—	—	Castledonate No. 257	Mixed.
	3	—	—	—	—	—	—	—	—	".....	—
25. Umtata, Cape Province	Pasture 1	-38	-20	-55	-96	-24	6-4	—	—	Farm Haddon.....	Mature.
	2	-36	-14	-88	-48	-68	4-2	—	—	Castledonate No. 257	Mixed.
	3	—	—	—	—	—	—	—	—	".....	—
26. Umtata, Cape Province	Soil... 1	-085	-092	-0105	-0307	-0087	-042	—	—	Zwartfontein.....	Mixed, mainly mature.
	2	-00054	-250	-030	-0307	-0087	-092	—	—	Mimosa Farm.....	Mixed.
	3	-00107	-133	-043	-0113	-0087	—	—	—	".....	"
27. Umtata, Cape Province	Pasture 1	-51	-16	-80	-15	-23	3-5	—	—	Zwartfontein.....	Mixed, mainly mature.
	2	-25	-29	-14	-20	-41	4-0	—	—	Mimosa Farm.....	Mixed.
	3	-39	-10	-58	—	-25	3-0	34-8	60-1	".....	"
28. Umtata, Cape Province	Soil... 1	-168	-080	-015	-015	-0017	-125	—	—	Xura Area.....	Mixed, mainly mature.
	2	-115	-086	-0186	-0120	-0035	-316	—	—	Xura Area.....	Mixed.
	3	-36	-29	-35	-38	-18	4-0	—	—	".....	Mixed.
29. Umtata, Cape Province	Pasture 1	-12	-19	-31	—	-35	3-7	29-6	61-2	Xura Area.....	Mixed.
	2	—	—	—	—	—	—	—	—	".....	—
	3	-27	—	—	—	—	—	—	—	".....	—

TABLE 1—(continued).

6

District and Number of Survey.	P ₂ O ₅ .	CaO.	MgO.	K ₂ O.	Na ₂ O.	Cl.	N.	Fibre.	Carb. + Ether Sol. Ext.	Farm.	Nature of Pasture.
25. Queenstown, Cape Province	Soil... 1 2 3	.286 .101 .132	.184 .050 .051	.0825 .0283 .0365	.0580 .0057 .0034	.0017 Nil .0017	.075 .0644 .081	— — —	— — —	Endwell..... Mt. Hupeley..... "	— — —
	Pasture 1 2 3	.51 .30 .21	.27 .17 .10	1.33 1.23 .91	.13 .10 —	.38 .27 .20	5.1 6.6 5.9	— — 27.0	— — 62.9	Endwell..... Mt. Hupeley..... "	Mixed, mainly mature. Mixed, practically all green. Mixed.
	Soil... 1 2 3	.132 — —	Nil — —	.0145 — —	.0088 — —	Nil — —	.1246 — —	— — —	— — —	Craddock Place..... "	— — —
26. Port Elizabeth, Cape Province	Pasture 1 2 3	.57 .91 —	.18 .23 —	1.80 1.43 —	.47 .65 —	.83 1.00 —	— 9.8 —	— 29.6 —	— 57.2 —	Craddock Place..... "	Green. Green grass and shrubs.
	Soil... 1 2 3	.051 .035 —	Nil Nil —	.0128 .0127 —	.0083 .0068 —	.0083 .021 —	.042 .0378 —	— — —	— — —	Woodlands..... "	— — —
	Pasture 1 2 3	.58 .21 .30	.23 .13 .17	.96 .70 .96	.23 .28 .19	.21 .29 .36	6.2 4.6 7.2	— — 25.6	— — 61.6	Woodlands..... Lombardspost..... "	Mixed, mainly mature. Mixed, practically all green.
28. Albany, Cape Province	Soil... 1 2 3	.174 .858 .544	.076 .276 .169	.0415 .1148 .082	.0214 .0244 .050	.0035 .0017 .089	.078 .063 .232	— — —	— — —	Peacock..... Kingston..... "	— — —
	Pasture 1 2 3	1.70 1.44 2.59	.62 .43 .47	2.67 1.8 1.04	1.04 .73 .57	.74 .53 .57	14.6 7.7 10.4	— — 24.4	— — 63.2	Peacock..... Kingston..... "	Leaves of shrubs. Green grass and shrubs. "
	Soil... 1 2 3	.438 .481 .610	.157 .150 .160	.0481 .0715 .067	.0294 .0273 .026	.0052 .0157 Nil	.115 .0966 .097	— — —	— — —	Prinstone..... "	— — —
29. Bedford, Cape Province	Pasture 1 2 3	.42 .36 .87	.27 .20 .32	1.27 1.31 1.73	.57 .56 .28	.48 .87 .56	4.5 6.0 7.4	— — 31.5	— — 57.9	" " "	Mixed, practically all mature. Mixed grass and shrubs. Mixed.

TABLE 1—(continued).

District and Number of Survey.	P ₂ O ₅ .	CaO.	MgO.	K ₂ O.	Na ₂ O.	Cl.	N.	Fibre.	Carb. + Ether Ext.	Farm.	Nature of Pasture.
30. Middelburg, Cape Province	Soil... 1 2 3	·668	·103	·0600	·0109	·0035	—	—	—	Allandale.....	—
	Pasture 1 2	— ·56	— ·18	— 1·22	— ·36	— ·21	— 6·6	—	—	Allandale.....	Mixed, mainly green grass and shrubs.
	3	·57	·19	1·10	·09	·15	6·3	28·9	58·9	"	Mixed, mainly green.
31. Barkly West, Cape Province	Soil... 1 2 3	·031	Nil	·0203	·0097	·0175	·0966	—	—	Greefputs.....	—
	Pasture 1 2	— ·28	— ·13	— ·92	— ·14	·17	5·4	—	—	Greefputs.....	Mixed, mainly mature. Old dry mature.
	3	·27	·26	·48	·01	·07	2·9	37·3	58·1	"	—
32. Blesfontein, Orange Free State	Soil... 1 2 3	·390 ·288	·258 ·155	·013 ·0324	·017 ·0102	·0017 ·0017	·076 ·077	—	—	Bissshops Glen..... Glen Shields.....	—
	Pasture 1 2	·63 ·38	·47 ·30	·41 1·30	·34 ·26	·11 ·25	5·8 5·2	—	—	"	Mixed, mainly mature. Mixed grass and shrubs.
	3	·66	·29	·76	·13	·19	5·6	30·9	60·9	"	" ..
33. Kroonstad, Orange Free State	Soil... 1 2 3	·171	·067	·0391	·0055	Nil	·0896	—	—	Naseby Thorns.....	—
	Pasture 1 2	— ·31	— ·12	— 2·20	— ·26	·36	3·6	—	—	"	—
	3	·16	·15	·41	·03	·09	3·4	33·1	59·9	Naseby Thorns.....	Mixed, mainly mature. Mixed, practically all mature.
34. Vryburg, Cape Province	Soil... 1 2 3	·287 ·188	·059 ·054	·032 ·0268	·0115 ·0073	Nil Nil	·048 ·0476	—	—	Content.....	—
	Pasture 1 2	— ·13	— ·10	— ·45	— ·02	·13	3·7	—	—	"	Mixed, practically all mature.
	3	·52	—	1·00	·09	·24	3·8	—	—	Content.....	Mixed.

TABLE 1—(continued).

District and Number of Survey.	P ₂ O ₅	CaO.	MgO.	K ₂ O.	Na ₂ O.	Cl.	N.	Fibre.	Carb. + Ether Sol. Ext.	Farm.	Nature of Pasture.
35. Vryburg, Cape Province	Soil... 1 2 3	.130 .197	.056 .087	.026 .0112	.0058 .0188	.0035 Nil	.042 -.0476	—	—	Armoedsvakte,....	—
	Pasture 1	.69	.29	1.24	.40	.16	6.9	—	—	" "	—
	Pasture 2 3	.48	.18	1.02	.11	.18	4.1	—	—	Armoedsvakte,....	Mixed, mainly mature.
36. Bethelhem, Orange Free State	Soil... 1 2 3	.106	.047	.0258	.0133	Nil	.081	—	—	The Outlook,.....	—
	Pasture 1	.40	.15	.78	.03	.15	4.2	—	—	The Outlook,.....	Mixed, mainly mature.
	Pasture 2 3	.24	.09	.52	—	.04	3.7	35.6	56.4	The Outlook,.....	Mixed, practically all mature.
37. Matelking, Cape Province	Soil... 3 { Pasture,....	.036 .25	Trace .16	.0151 .67	.0025 .03	Nil .12	.028 2.5	44.7	50.3	Gemsbokspan,.....	Old dry mature.
38. Marico, Transvaal	Soil... 3 { Pasture,....	.563 .34	.331 .23	.0216 1.77	.0139 .14	Nil .31	.095 5.9	33.4	57.2	Olfantsvllei,.....	Mixed, mainly mature.
	Soil... 3 { Pasture,....	.403 .35	.436 .21	.0167 .19	.0066 .01	Nil .03	.118 2.2	37.8	—	Melrose Farm,.....	Mixed, practically all mature.
39. Rustenburg, Transvaal	Soil... 3 { Pasture,....	.22	.11	.45	.03	.11	3.1	34.2	60.6	Rustfontein,.....	Mixed, practically all mature.
	Soil... 2 { Pasture,....	.168 .57	.042 .37	.0371 2.4	.0079 .37	Nil .65	.1316 6.2	—	—	Elandsdraai,.....	Mixed, mainly mature.
42. Witbank, Transvaal	Soil... 2 { Pasture,....	.024 .17	Trace .13	.0122 .24	.0045 .02	Nil .03	.0546 1.9	—	—	Sukkerbosrand,.....	Mixed, practically all mature.
	Soil... 2 { Pasture,....	.056 .14	Nil .08	.0139 .84	.0075 .20	.005 .30	.063 3.2	—	—	Hartbeest,.....	Mixed, mainly mature.
43. Worcester, Cape Province	Soil... 2 { Pasture,....	.300 .60	.131 .24	.0466 1.71	.0343 .42	.0087 .58	.084 .70	—	—	De Brak No. 1064, ..	Mixed.
44. Pongletersrust, Transvaal	Soil... 1 { Pasture,....	.022 .45	.030 .36	.0087 .19	.0109 .09	Nil .24	.018 6.1	—	—	Viesgat,.....	Mixed, mainly mature.
45. Waterberg, Transvaal	Soil... 1 { Pasture,....	.857 .38	.160 .16	.0762 .87	.0504 .08	.017 .20	.136 3.5	—	—	Quest,.....	Mixed, practically all mature.
46. Uthombo, Natal	Soil... 1 { Pasture,....	.419 .54	.109 .21	.0282 1.02	.022 .29	Nil .26	.221 5.8	—	—	M'Kuzi Settlement, ..	Mixed.
47. East London, Cape Province	Soil... 1 { Pasture,....									Emerald Vale,.....	Mixed.
										Umdaung,.....	—

TABLE 1—(continued).

District and Number of Survey.	P ₂ O ₅	CaO	MgO	K ₂ O	Na ₂ O	Cl	N	Fibre.	Carb. + Ether Sol. Ext.	Farm.	Nature of Pasture.
48. Robertson, Cape Province	1 { Soil. Pasture.021 .55	Nil .30	.011 .83	.006 .46	Nil .43	.039 8.5	—	—	St. Hilda,	Green grass and shrubs. —
49. George, Cape Province	1 { Soil. Pasture.090 .60	.061 .17	.012 .85	.0156 .23	.0035 .23	.116 4.9	—	—	Oaklands,	Green grass and shrubs. —
50. Bloemfontein, Orange Free State	1 { Soil. Pasture.084 .31	.070 .27	.023 .57	.0125 .03	Nil .12	.041 6.3	—	—	Holmesdale,	Mixed, practically all mature. —
51. Estcourt, Natal	1 { Soil. Pasture.	— .57	— .19	— .54	— .02	— .08	— 2.7	—	—	Kimbolton,	Mixed, practically all mature. —
52. Dundee, Natal	3 { Soil. Pasture.054 .27	.040 .17	.0279 .62	.0033 .03	Nil .12	.077 3.7	—	61.1	Halifax,	Mixed. —
53. Ladysmith, Natal	2 { Soil. Pasture.103 .25	.076 .13	.018 .48	.0077 .02	Nil .08	.1008 1.4	—	—	Honefarm,	Mixed, practically all mature. —
54. Impeddie, Natal	1 { Soil. Pasture.095 .35	.071 .12	.0206 .97	.0156 .37	.0052 .19	.280 4.6	—	—	Vandus,	Mixed. —
55. Camperdown, Natal	2 { Soil. Pasture.148 .30	.095 .12	.0187 .53	.0048 .07	Nil .15	.1386 4.3	—	—	Mountain View,	Mixed, mainly green. —
56. Richmond, Natal	3 { Soil. Pasture.378 .20	.152 .07	.0382 .44	.0089 .03	Nil .10	.279 3.7	—	54.2	Commisledrift,	Mixed. —
57. Bizama, Cape Province	2 { Soil. Pasture.191 .28	.102 .14	.010 .63	.015 .14	.0017 .38	.141 2.9	—	—	Commonage,	Mixed. —
58. Uitenhage, Cape Province	1 { Soil. Pasture.190 .39	.056 .25	.050 2.07	.038 .80	.014 .84	.154 10.2	—	—	Perseverance,	Mixed, practically all mature. —
59. Somerset West, Cape Province	1 { Soil. Pasture.028 .16	Nil .07	.014 .48	.0085 .11	Nil .24	.059 3.5	—	—	Lourensford,	Thick reedy grass, mixed, mainly mature. —
60. Melmsbury, Cape Province	2 { Soil. Pasture.0003 .14	.025 .10	.0076 .76	.0051 .29	.007 .40	.0098 5.6	—	—	Chatsworth,	Mixed. —
61. Tygerberg, Cape Province	3 { Soil. Pasture.0018 .09	.038 .22	.0095 .09	.0018 .53	.0105 1.10	.021 4.7	—	60.0	Welbeloed,	Old mature cyperaceae. —
62. Kimberley, Cape Province	1 { Soil. Pasture.0005 .07	.046 .17	.023 .80	.008 .07	.0017 .16	.028 3.4	—	—	Proogfontein,	Mixed, practically all mature. —

2. DISCUSSION OF RESULTS GIVEN IN TABLE 1.

(a) Explanation of Terms.

It should be mentioned that the terms used in the last column under nature of pasture in the above table will be employed throughout for this type of table and have been selected to designate the following: All the terms refer to grasses except in the few cases where "shrub" has been inserted. A grass consisting of apparently equal quantities of green and mature grass—mature indicating definitely not green—is named "mixed", while a mixture of green and mature grass with one kind definitely predominant is called "mixed, mainly mature" or "mainly green", as the case may be. If the sample consists almost exclusively of green or mature grass with only a sprinkling of the other, it is called "mixed, practically all mature" or "green" according to which grass is almost exclusively present. The remaining terms "old, dry mature" and "green" are self explanatory.

(b) Classification of Pasture.

The table given above reveals a number of very interesting points and throws a great deal of light on some of the pasture problems of the Union.

If the description of the pasture for mineral surveys 1 and 2 given in the last column be looked at it is seen that for both periods the grasses varied almost without exception between a mixture of green and mature grasses to one in which mature grasses definitely predominated (i.e. mixed, mainly mature). Mid-winter is in July, when heavy frosts have usually fallen, which would obviously further reduce the small percentage of green pasture if any is present at all, so that the analyses given for pasture in May are probably better than the figures for mid-winter.

The description of the pasture for October, 1931, is not normal for most years. Usually some rains have fallen and young grass has made its appearance so that "old dry mature", which often appears in the table under review, should perhaps be less in evidence in order to give a true conception of the usual state of the pasture in mid-spring. In 1931 a severe drought existed in a number of areas, as is distinctly indicated not only by the description of the pasture but by its chemical composition, and especially by its protein content. If the description of the pasture for the three surveys be taken as a whole, it becomes evident that for May, 1930, May, 1931, and October, 1931, there were very few outstanding differences between the proportions of green and mature grasses present and that with few exceptions the animals had to be content with the less palatable and less nutritious mature pasture in the absence of green feed. It will be interesting to note changes in the state of the pasture for surveys that have been made during other seasons of the year. These results will be published in due course.

(c) Crude Protein, Fibre and Carbohydrate plus Ether Soluble Extract.

The crude protein content of the grass samples collected during all three surveys was determined, while for the Third Survey, October, 1931, the analysis for fibre and carbohydrates plus ether

soluble extract was also undertaken. The crude fibre content appears to be high and is rarely below 30 per cent., while it remains on an average in the neighbourhood of 40 per cent. The carbohydrate plus ether soluble extract values are round about 55 per cent., and as the latter were invariably found to be below 5 per cent.—about 20 analyses were made—the carbohydrate fraction of the pasture was about 50 per cent. The values for protein are strikingly low; only in exceptional cases do the figures reach 10 per cent. while values less than two have been obtained and 3 and 4 per cent. are the rule rather than the exception. It must be remembered that the grasses, as already stated, varied between mixtures of about equal quantities of green and mature on the one hand and old dry mature grass on the other. The crude protein content of the pasture for the periods May, 1930, May and October, 1931, therefore applies to periods of winter grazing and drought and should not be taken as a general figure for South African grasses. However, the fact remains that growing sheep requiring approximately 18 lb. of digestible protein per day and consuming about 2·5 lb. of winter grazing per day would ingest not more than 0·09 lb. of crude protein on pasture such as most of that given in Table 1. The question of a probable protein deficiency in winter grazing and during early spring, if future surveys during these periods bear out the figures obtained for pasture in May and October, 1931, will be kept in view. It may be added that the nitrogen content of the soils is a good average and on the whole above 0·5 per cent., a figure usually indicative of the necessity of nitrogen fertilizers.

(d) *The Phosphorus Content of Pastures.*

The mineral content of the pastures is most striking in some respects. The values for phosphorus are extremely low on the whole and the often recorded fact that the phosphorus content of grasses decreases with the age of the grass has again been noted, mineral surveys one and two (May, 1930, and May, 1931, respectively) showing higher values than those obtained in October, 1931, in areas where drought existed, as a glance at Table 2, giving the monthly rainfall will reveal and where as a result no new grass was present. In any case, a comparison between the phosphorus content of the pasture in May and in October is hardly necessary as the figure for winter pasture is already so low in most cases that it denotes the presence of mature grasses mainly. Such a comparison is only interesting as it reveals the length of the period in 1931 that stock had to be content with pasture of low feeding value and phosphorus content. The deploring fact remains that in 1931 the pasture showed very little improvement in most areas as far as the phosphorus content was concerned from the beginning of winter until mid-spring. There are some exceptions naturally. Zoutpansberg, area 6, shows a rainfall in Table 2 of about an inch in August and the phosphorus content of the pasture was 44 per cent. in October instead of the low value of 16 in May. The figures for area 1—Middelburg, Transvaal—are not comparable, as different farms were used for the three surveys. Areas 4, 5, 9, 10 show a decided drop and Table 2 shows no rain in spring. Areas 15, 16, 17 and 18 show high rainfalls, although, except 17, which has had the least rain, the phosphorus remains low. This observation appears to lend strength to the contention of Van Wyk (1932) that heavy rains rob

plants of their phosphorus by leaching. Area 20 shows appreciably higher phosphorus in October after a heavy late winter rain. It must be remembered that comparisons are made only where the same farm was used for both the surveys in 1931, while mineral survey 1 in May, 1930, provides an additional figure for a winter survey. A study of the remaining areas continues to show a large number of low values; as a matter of fact, of the total number of 115 analyses made for phosphorus for the three surveys, 72 show a value lower than .2 per cent., while 29 lie between .2 and .4 per cent., and only 14 above the fair average of .4 per cent. P_2O_5 . It must be added that in areas 26 (Port Elizabeth), 28 (Albany), 29 (Bedford), fairly high to high values were obtained for phosphorus for all three surveys, and that in practically all cases shrubs which are admittedly higher in phosphorus than grasses were present in the samples analysed.

(e) *The Phosphorus Content of the Pastures in Relation to the Phosphorus Content of Soils.*

The figures for the available phosphorus in soils with very few exceptions are exceedingly low on the whole. Values lower than approximately .005 per cent. are taken to indicate phosphorus deficiency, and it is interesting to note that the three areas 26, 28 and 29 mentioned above (where high values for P_2O_5 in pasture were obtained) are all, except one (where only one survey was made) well above the borderline of phosphorus deficiency. Area 6 (Zoutpansberg, Transvaal) shows very high available soil phosphorus, although its pasture in May, 1931, was very low. A correlation of the phosphorus content of the soil and the pasture can hardly be expected to show in Table 1, except in a very general way, such as extremely low phosphorus in soils generally and low in the herbage on the whole. It is very doubtful whether the phosphorus content of the pasture collected at various stages of growth, growing on different types of soil, subjected to different climatic conditions and not composed of the same kinds of grasses, will show any correlation with the phosphorus content of the soil.

At all events such a correlation is not apparent from a study of Table 1, except in the few areas already mentioned, where high soil phosphorus apparently resulted in a high figure for phosphorus in pasture. To complete the list all the areas showing enough soil phosphorus (i.e. above .005 per cent.), except No. 6, could be added. In all cases fairly high to high values were obtained for the phosphorus content of the pasture. The exception—area No. 6—is interesting in that for the third survey the correlation between high phosphorus in the soil and high phosphorus in the pasture does exist but not for the second survey, when, incidentally, the pasture consisted almost exclusively of mature grasses low in phosphorus. The stage of growth of plants, which is determined to a large extent by climatic conditions, is undoubtedly an important factor in determining their phosphorus content, for it is seen in Table 1 that green pasture invariably shows a satisfactory value for phosphorus independent of the phosphorus content of the soil. Furthermore, a perusal of the results showing low soil phosphorus, i.e. below .005 per cent., indicates that values of phosphorus in the pasture vary from extremely low figures,

viz., .07 per cent for mature and old dry grasses to medium and high values, viz., .46 per cent. for green pasture. The phosphorus content of the herbage in these cases apparently bears no relation to the soil phosphorus.

In conclusion it may be added that it is not the intention to prove an existing correlation between the phosphorus content of soils and the pasture growing upon them from the figures presented in Table 1. For that purpose climatic conditions, stage of growth and differences in plant species would have to be equalized, which obviously could not have happened in the results under discussion. The main issue, however, remains that the soil analyses provide proof of an acute phosphorus deficiency in most areas, while the pasture analyses largely corroborate this view with the indication that climatic conditions and stage of growth are factors which influence very largely or may even completely change the picture of the phosphorus content of the pastures.

The possibility of a correlation of the results of soil analyses with those of pasture and blood analyses are dealt with from a different angle on page 569.

(f) CaO, MgO, K₂O, Na₂O, AND Cl.

Compared with European figures the calcium content of South African pastures in winter and spring, 1931, were low. Even soils high in calcium apparently had little effect on the calcium content of the herbage. For instance, pasture on a soil containing .4 per cent. CaO showed .41 per cent. CaO, whereas one on a soil containing only .078 per cent. showed .44 per cent. CaO. There appears to be less variation in the calcium content of the pasture of different ages, although it must be added that the values taken at random are considerably more constant than in the case of phosphorus. The calcium content of shrubs, like their phosphorus content, is higher generally than in grasses. Emphasis must be laid again on the fact that the analyses represent pastures during the driest and least abundant season when the feeding value and quality are at their lowest as a consideration of the protein content reveals. It would be better to stress the length of time that only poor pasture was available in 1931 than the unfavourable comparison of these figures with the values given by Orr (1929) for cultivated or even "all grazed" natural pasture as the figures given in Table 1 represent the composition mainly of fully grown mature pasture which is admittedly less nutritious than younger herbage or even than the portion which would be selected by the grazing animal.

The values for magnesium given in Table 1 require little comment as quite a fair average is apparently maintained even in old mature herbage. The amount is probably more than sufficient in all cases for the animals' need. The values lie between .11 and .32 for grasses, while a few higher figures in samples consisting of a mixture of grasses and shrubs were obtained.

Potassium varies between .22 and 2.6 in pastures with an average round about 1 per cent., while the available potassium in soils does not show anything like the variation that the lime content does.

TABLE 2.

RAINFALL IN INCHES.
February–September, 1931.

District.	Farm.	Feb.	March.	April.	May.	June.	July.	Aug.	Sept.
1. Middelburg, Transvaal.....	Aberdeen No. 291.....	1.96	1.65	Nil	—	—	1.96	—	—
2. Krugersdorp, Transvaal.....	Roodepoort No. 8.....	—	1.28	—	Nil	Nil	1.32	—	—
3. Barberton, Transvaal.....	Hartebeesfontein.....	3.74	1.36	2.52	—	—	1.16	—	—
4. Lydenburg, Transvaal.....	Brooklyn.....	5.80	2.4	2.0	—	—	—	—	—
5. Marico, Transvaal.....	De Grootboom No. 214.....	3.34	1.97	1.15	Nil	—	—	—	—
6. Zoutpansberg, Transvaal.....	Kaaplaats No. 97.....	3.13	1.43	3.34	Nil	—	—	—	—
7. Potgietersrust, Transvaal.....	Govt. Ranching Station.....	.60	1.35	1.35	Nil	—	—	—	—
8. Piet Retief, Transvaal.....	Mooglegelen No. 635.....	2.27	3.50	1.34	Nil	—	—	—	—
9. Pietersburg, Transvaal.....	Derby No. 56.....	1.71	3.47	3.05	Nil	—	—	—	—
10. Potchefstroom, Transvaal.....	Wildebeesfontein No. 89.....	1.61	5.31	1.55	Nil	—	—	—	—
11. Ermelo, Transvaal.....	Mimosa Park.....	2.01	1.68	2.82	Nil	—	—	—	—
12. Ikopo, Natal.....	Lelefontein.....	2.56	1.97	4.02	Nil	—	—	—	—
13. Vryheid, Natal.....	Stainton.....	4.2	4.6	2.01	Nil	—	—	—	—
14. Nongoma, Natal.....	Bergendal.....	2.95	1.10	2.59	Nil	—	—	—	—
15. Phinetown, Natal.....	Zulu National Instit.....	2.46	2.82	.80	Nil	—	—	—	—
16. Eshowe, Natal.....	Zeekoegat.....	1.40	6.38	1.51	Nil	—	—	—	—
17. Greytown, Natal.....	Marianhill.....	—	—	—	Nil	—	—	—	—
18. Port Shepstone, Natal.....	Lots 45/48, Entumeni.....	3.77	3.02	1.86	Nil	—	—	—	—
19. Butterworth, Cape Province.....	Area No. 24.....	5.58	3.75	1.89	.55	1.62	8.26	.36	1.37
20. Kingwilliamstown, Cape Province.....	Melbourne.....	2.96	10.11	1.60	.25	.14	1.92	1.65	2.42
21. Kokstad, Cape Province.....	Woodlands.....	1.75	2.9	1.02	Nil	—	—	—	—
22. Komgha, Cape Province.....	Rockdale.....	3.50	3.20	3.10	.07	.42	—	—	—
23. Umkata, Cape Province.....	Koppieskraal.....	3.56	5.10	1.84	Nil	—	—	—	—
24. Lusikiski, Cape Province.....	Castledondale No. 257.....	9.49	2.2	1.92	.05	.13	7.47	.11	.25
25. Queenstown, Cape Province.....	Mimosa Farm.....	10.63	5.95	.85	.21	Nil	14.14	.84	1.20
26. Port Elizabeth, Cape Province.....	Xura Area.....	.86	3.39	1.32	.80	Nil	6.56	Nil	—
27. Bathurst, Cape Province.....	Mt. Hupeley.....	.46	2.27	1.32	.23	1.22	3.95	1.24	4.64
28. Albany, Cape Province.....	Cradock Place.....	—	1.59	5.54	.67	.50	3.83	.22	2.84
29. Bedford, Cape Province.....	Lombardspost.....	.24	1.22	2.33	.05	.07	2.25	Nil	1.09
30. Middelburg, Cape Province.....	Kingston.....	Nil	3.36	Nil	Nil	.28	3.46	.53	Nil
31. Barkly West, Cape Province.....	Primestone.....	.92	2.59	1.49	Nil	.08	1.58	Nil	Nil
32. Bloemfontein, Orange Free State.....	Allandak.....	2.93	2.55	1.15	Nil	.005	—	—	—
33. Kroonstad, Orange Free State.....	Greeputs.....	2.89	1.73	4.55	Nil	Nil	1.48	.11	Nil
34. Vryburg, Cape Province.....	Glen Shields.....	2.14	2.95	2.35	Nil	—	.75	—	—
35. Bethlehem, Orange Free State.....	Naseby Thoris.....	.62	1.87	3.31	Nil	—	—	—	—
36. Mafeking, Cape Province.....	Content.....	2.50	3.53	3.82	Nil	—	—	—	—
37. Camperdown, Natal.....	Armoedsvlakte.....	3.66	.59	2.62	Nil	—	—	—	—
	Outlook.....	.79	.85	1.85	Nil	—	—	—	—
	Gemsbokpan.....	—	—	—	Nil	—	—	—	—
	Mountain View.....	—	—	—	Nil	—	—	—	—

It appears that the potassium content of grasses decreases with age, although the values for soil potassium in areas 28 and 29 tend to show a distinct correlation between high soil potassium with high pasture values. However, this correlation is imperfect for low soil potassium shows high values in pastures in a number of instances, although the opposite has not been observed in a single case.

Sodium in pasture appears to vary without regard to the sodium content of the soil, although it does seem to decrease definitely with the age of the grass. The wide variation in the sodium content of pastures is noteworthy, values as low as .01 per cent. having been obtained, while .3 is not uncommon and several high values round about .7 have been registered.

The remaining element, viz., chlorine, shows great variation both in soils and in pasture. Here again the chlorine content of pasture is seen to decrease definitely with the age of the plant. The few figures available in Table 1 for chlorine in green pasture are all high, while old dry mature grass shows greatly decreased values.

Finally, it must be mentioned that the herbage of which the chemical composition is present in Table 1 obviously does not necessarily represent the portion actually eaten by animals, for it is known that certain parts are grazed in preference to others, especially during winter, when grazing is mainly mature and frequently hard and fibrous.

Discussion.

A glance at Table 3 indicates wide variations in the chemical composition of the same species from different areas, while the last column of the table shows that the individual species were analysed at various stages of growth. In other words, climatic conditions, including differences in soil conditions, produced different stages of growth in the same species, causing in their turn variations in the chemical composition of such species. Species one—*Themeda triandra*—shows samples consisting mainly of green grass on the one hand and practically all mature on the other. The major proportion of the samples were composed of mature grasses, due to lateness of the time of sampling—May, 1931—and, as the description of the samples in the last column reveals, with generally low values for P, Na, chlorine and protein. The same grass—No. 10 in the table—in October, 1931, was obtained from 5 of the areas included in the May survey, and a comparison of the chemical composition at the two periods is interesting:—

Locality.	Survey.	P ₂ O ₅ .	CaO.	MgO.	K ₂ O.	Na ₂ O.	Cl.	Protein.
Pietersburg...	2	.14	.24	.12	.71	—	.20	2.1
	3	.14	.28	.12	.48	.01	.13	2.0
Potchefstroom.	2	.11	.36	.25	.74	—	.18	2.1
	3	.07	.17	.07	.31	.01	.06	2.2
Kokstad.....	2	.18	.28	.13	.75	—	.16	3.9
	3	.16	.38	.26	.59	—	.19	3.7
Kroonstad....	2	.3	.31	.08	.81	—	.16	2.5
	3	.12	.24	.16	.44	—	.04	2.2
Ermelo.....	2	.11	.36	—	.71	—	.19	2.4
	3	.3	.34	.24	1.23	—	.36	8.1

3. THE CHEMICAL COMPOSITION OF THE SAME SPECIES OF GRASSES FROM DIFFERENT AREAS.

TABLE 3.

MINERAL SURVEY II, MAY, 1931.

Value in gm. per 100 gm. dry material.

Species.	Locality.	P ₂ O ₅ .	CaO.	MgO.	K ₂ O.	Na ₂ O.	Cl.	Protein.	Nature of Grass.
<i>Themeda triandra</i> .	Aberdeen No. 291, Middelburg, Transvaal	0.14	0.25	0.16	0.92	0.04	0.23	3.7	Mixed mainly green.
	De Groothoorn No. 214, Lydenburg	0.11	0.35	0.10	0.97	0.07	0.25	2.1	Mixed, practically all mature.
	Moogleggen No. 635, Potgietersrus	0.18	0.39	0.16	0.87	0.05	0.13	2.0	Mixed, mainly mature.
	Wilbeesfontein, Pietersburg	0.14	0.24	0.12	0.71	0.05	0.20	2.1	Mixed, mainly mature.
	Mimosa Park, Potchefstroom	0.11	0.36	0.25	0.74	0.08	0.18	2.1	Mixed, mainly mature.
	Stainton, Ixopo,	0.14	0.25	0.25	0.64	0.11	0.22	2.8	Mixed, mainly green.
	Koppieskraal, Koksstad,	0.18	0.28	0.13	0.75	0.14	0.16	3.9	Mixed.
	Castledendale No. 257, Komgha	0.03	0.24	0.10	0.51	0.04	0.12	2.8	Mixed, mainly mature.
	Woodlands, Bathurst,	0.23	0.31	0.13	0.55	0.14	0.17	5.0	Mixed, mainly mature.
	Kingston, Albany,	0.39	0.32	0.10	0.75	0.03	0.17	3.3	Mixed, mainly mature.
	Naseby Thoras, Kroonstad, ..	0.30	0.31	0.08	0.81	0.06	0.16	2.5	Mixed, mainly mature.
	Content, Vryburg,	0.14	0.45	—	0.97	0.10	0.24	3.4	Mixed.
	Leliefontein No. 23, Ermelo.	0.11	0.36	—	0.71	0.09	0.19	2.4	Mixed.
	Derby No. 56, Piet Retief, ..	0.14	0.41	0.15	0.84	0.03	0.28	2.3	Mixed, mainly mature.
<i>Hypparrhenia hirta</i> .	Bergendal, Vryheid,	0.03	0.21	0.20	0.79	0.11	0.24	1.8	Mixed, practically all mature.
	Zulu National Training Inst.—Nongoma,	0.11	0.21	0.25	0.48	0.06	0.23	3.5	Mixed, practically all mature.
	Lots Nos. 45/48, Entumeni, Eshowe	0.11	0.34	0.13	0.55	0.04	0.10	2.5	Mixed, practically all mature.
	Area No. 24, Umvoti, Natal	0.16	0.41	0.10	0.85	0.03	0.18	1.8	Mixed, mainly mature.
	Koppieskraal, Koksstad,	0.25	0.29	0.32	0.63	0.07	0.17	2.5	Mixed.
	Mimosa, Umtata,	0.11	0.35	0.17	0.74	0.05	0.22	2.3	Mixed, mainly mature.
	Commonage, Bizana,	0.18	0.43	0.18	0.60	0.10	0.35	2.9	Mixed.

TABLE 3—(continued).

Species.	Locality.	P ₂ O ₅ .	CaO.	MgO.	K ₂ O.	Na ₂ O.	Cl.	Protein.	Nature of Grass.
<i>Panicum maximum</i>	Govt. Ranching Station, Zoutpansberg	0.21	0.55	0.35	1.58	0.25	0.67	4.3	Mixed, mainly mature.
	Zulu National Training Inst., Nongoma	0.21	0.46	0.43	0.95	1.00	0.70	6.9	Mixed.
	Kingston, Albany..... Elandskraal, Pretoria.....	0.94 0.53	0.38 0.63	0.28 —	1.11 2.54	0.47 0.47	0.52 0.50	5.2 3.7	Mixed. Mixed, practically all mature.
<i>Eragrostis plana</i> ...	Derby No. 56, Piet Retief..	0.09	0.34	0.05	0.57	0.09	0.22	2.3	Mixed, mainly mature.
	Stanton, Ixopo.....	0.14	0.24	0.15	0.71	0.08	0.23	3.2	Mixed, mainly green.
	Koppieskraal, Kokstad..... Woodlands, Bathurst.....	0.23 0.14	0.29 0.21	0.10 0.10	0.87 0.41	0.21 0.12	0.26 0.12	3.9 3.1	Mixed, mainly mature. Mixed, mainly mature.
<i>Chrysosoma tenuifolia</i>	Kingston, Albany.....	0.66	0.70	0.30	2.4	0.78	0.78	9.2	Shrub.
	Allandale, Middelburg, C.P..	0.27	0.80	0.25	1.74	0.62	0.19	7.0	Shrub.
	Glen Shields, Bloemfontein..	0.73	0.84	0.56	2.3	—	0.43	13.5	Shrub.
<i>Rhynchosyrum roseum</i>	Zulu National Training Inst., Nongoma	0.16	0.55	0.42	0.50	0.08	0.36	3.7	Mixed, mainly mature.
	Greepputs, Barkly West.....	0.32	0.29	0.23	1.69	0.32	0.29	6.0	Mixed, mainly green.
	Armoedsvlakte, Vryburg....	0.18	0.55	0.32	1.11	0.13	0.15	5.0	Mixed, mainly mature.
<i>Eragrostis atherstonesi</i>	Mimosa Park, Potchefstroom	0.14	0.38	0.18	0.73	0.03	0.22	3.1	Mixed, mainly green.
	Allandale, Middelburg, C.P..	0.57	0.52	0.20	1.11	0.24	0.25	6.5	Green.
	Greepputs, Barkly West.....	0.14	0.21	0.10	0.68	0.03	0.12	4.7	Mature.

TABLE 3—(continued).

3

Species.	Locality.	P ₂ O ₅ .	CaO.	MgO.	K ₂ O.	Na ₂ O.	Cl.	Protein.	Nature of Grass.
<i>Chloris virgata</i> ,....	Zulu National Training Inst., Nongoma	0.25	0.45	0.27	0.68	0.94	1.44	6.8	Mixed.
	Glen Shields, Bloemfontein...	0.30	0.32	0.15	1.79	0.32	0.41	3.3	Mixed, practically all mature.
	Elandsdraai, Pretoria.....	0.34	0.66	—	2.88	0.50	1.07	4.6	Mature.
<i>Aristida junceaformis</i>	Zeekoegat, Pinetown.....	0.11	0.20	0.08	0.35	0.08	0.16	2.9	Mixed, mainly mature.
	Lots Nos. 45/48, Entumeni, Eshowe	0.09	0.20	0.07	0.33	0.06	0.11	3.1	Mixed, mainly mature.
	Melbourne, Port Shepstone..	0.09	0.26	0.10	0.25	0.07	0.09	2.8	Mixed, practically all mature.
	Hartebeest, Worcester.....	0.14	0.14	0.05	0.62	0.08	0.12	3.4	Mixed, mainly mature.
	Woodlands, Bathurst.....	0.18	0.21	0.12	0.41	0.14	0.29	4.2	Mixed, mainly mature.
	Leliefontein No. 25 Ermelo..	0.16	0.18	—	0.35	0.01	0.05	4.6	Mixed, practically all mature.
MINERAL SURVEY III, OCTOBER, 1931.									
Species.	Locality.	P ₂ O ₅ .	CaO.	MgO.	K ₂ O.	Na ₂ O.	Cl.	Protein.	Nature of Grass.
<i>Themeda triandra</i> ..	Hartebeestfontein, Krugers- dorp	0.09	0.52	0.15	0.39	0.01	0.06	4.3	Mature.
	Barberton.....	0.32	0.31	0.17	0.80	0.01	0.16	3.6	Mixed, practically all mature.
	Kaaplaats, Marico.....	0.09	0.28	0.13	0.25	0.03	0.07	2.5	Old, mature.
	Gemsbokpan, Mafeking.....	0.07	0.20	0.14	0.59	0.03	0.16	1.6	Old, mature.
	Derby No. 56, Piet Retief..	0.14	0.34	0.12	0.42	0.03	0.13	1.7	Mixed, practically all mature.
	Wildebeestfontein, Pieters- burg	0.14	0.28	0.12	0.48	0.01	0.13	2.0	Mixed, mainly mature.
	Mimosa Park, Potchefstroom	0.07	0.17	0.07	0.31	0.01	0.06	2.2	Old, mature.
	Rock Dale, Kingwilliamstown	0.37	0.38	0.20	1.28	0.19	0.48	10.0	Green.
	Koppieskraai, Kokstad.....	0.16	0.38	0.26	0.52	0.03	0.19	3.7	Mixed, mainly mature.
	Mimosa Farm, Umata.....	0.18	0.45	0.11	0.65	0.04	0.17	3.4	Mixed.
	Primstone, Bedford.....	0.30	0.46	0.16	0.31	0.12	0.23	6.6	Mixed.
	Greefputs, Barkly West.....	0.05	0.20	0.08	0.46	0.01	0.08	1.7	Old, mature.
	Glen Shields, Bloemfontein..	0.09	0.24	0.12	0.50	0.03	0.08	2.8	Mixed, practically all mature.
	Naseby Thomas, Kroonstad..	0.12	0.24	0.16	0.44	0.03	0.06	2.2	Mixed, mainly mature.
	The Outlook, Bethlehem....	0.14	0.35	0.15	0.45	0.01	0.06	4.3	Mixed, mainly mature.
	Leliefontein, Ermelo.....	0.30	0.34	0.24	1.23	0.04	0.36	8.1	Green.

TABLE 3—(continued).

4

Species.	Locality.	P ₂ O ₅ .	CaO.	MgO.	K ₂ O.	Na ₂ O.	Cl.	Protein.	Nature of Grass.
<i>Cynodon dactylon</i> ..	Mimosa Park, Potchefstroom Halifax, Dundee..... Primstone, Bedford.....	0.03 0.27 0.73	0.34 0.45 0.41	0.11 0.27 0.17	0.27 1.47 1.61	0.01 0.20 0.46	0.05 0.29 0.53	3.6 8.4 11.0	Mixed, mainly mature, Green. Mixed, mainly green.
<i>Eragrostis curvula</i> ..	Derby No. 56, Piet Retief.. Mimosa Farm, Umtata.....	0.11 0.21	0.31 0.29	0.11 0.08	0.40 0.57	0.01 0.04	0.11 0.24	2.1 4.1	Mixed, practically all mature, Mixed.
<i>Chrysocoma tennifolia</i>	Primstone, Bedford..... Allandale, Middelburg, C.P.. Glen Shields, Bloemfontein..	0.37 0.23 0.71	1.71 0.73 1.36	0.43 0.40 0.61	2.31 1.84 1.08	0.42 0.16 0.44	0.85 0.20 0.43	8.5 6.9 8.6	Shrub. Shrub. Shrub.
<i>Heteropogon contortus</i>	Wilbeesfontein, Pieters- burg Greefputs, Barkly West.....	0.03 0.05	0.24 0.24	0.15 0.27	0.36 0.52	0.01 0.01	0.11 0.06	2.7 2.5	Mixed, mainly mature, Old, mature.
<i>Sporobolus indicus</i> .	Barberton..... Rockdale, Kingwilliamstown.	0.34 0.37	0.28 0.32	0.15 0.18	0.32 1.27	0.04 0.12	0.20 0.44	3.6 8.0	Mixed, mainly green, Green.
<i>Aristida junceaformis</i>	Marianhill, Pinetown..... Lots Nos. 45/48, Entuneni, Eshowe	0.14 0.14	0.22 0.21	0.03 0.06	0.48 0.38	0.04 0.03	0.16 0.12	3.6 4.1	Mixed, mainly mature, Mixed, mainly green.

The essential facts observed in Table 1 again stand out:—

- (1) In all cases except Ermelo when the sample consisted of green grass entirely, the phosphorus content of the pasture decreased from May to October, i.e. as the pasture became older, for drought existed and no new growth took place.
- (2) K, Na, Cl, and to a less extent protein, show the same decrease in values from May to October.
- (3) Calcium and magnesium showed variations, but no definite drop or rise.
- (4) Ermelo grass in October was quite green and showed an increase for K, Na, Cl and protein when compared with the analyses in May.

It appears that climate is the determining factor of a poor analysis or otherwise of a species of grass collected from several areas, which means that it has grown under different conditions. Rain or drought largely determines the stage of growth at any particular period, which in its turn determines the composition as regards P, K, Na, Cl and protein.

The composition of species No. 2—*Hyparrhenia hirta*—shows a number of similarities to *Themeda triandra*. The samples analysed were practically all mature and the values for P, K, Na, Cl and protein even lower than for *Themeda triandra*. Both high and low values for phosphorus were obtained for *Panicum maximum*. As a matter of fact the analyses of this species from all four areas indicate that it compares favourably in composition with other grasses given in the table. On the whole it must be said that with the exception of *Panicum maximum* and the shrub *Chrysocoma tenuifolia* the grasses given in Table 2 reveal very poor winter feeding. The analyses for October, 1931, largely corroborate this view, and it will be interesting to follow the seasonal composition of these grasses for later surveys.

4. THE CHEMICAL COMPOSITION OF DIFFERENT SPECIES OF GRASSES

FROM THE SAME AREA.

TABLE 4.

MINERAL SURVEY II, MAY, 1931.

Area.	Species.	P ₂ O ₅ .	CaO.	MgO.	K ₂ O.	Na ₂ O.	CL.	Protein.	Nature of Grass.
Govt. Ranching Station, Zoutpansberg	<i>Panicum maximum</i>	0.21	0.55	0.35	1.58	0.25	0.67	4.3	Mixed, mainly mature.
	<i>Aristida plumosa</i>	0.18	0.36	0.08	0.64	0.07	0.13	4.3	Mature.
	<i>Eneupogon mollis</i>	0.14	0.45	0.15	1.92	0.07	0.49	4.0	Mixed, practically all mature.
	<i>Pennisetum ciliare</i>	0.14	0.48	0.17	1.02	0.05	0.40	3.8	Mixed, practically all mature.
Moogelegen No. 635, Potgietersrust	<i>Themeda triandra</i>	0.18	0.39	0.16	0.87	0.05	0.13	2.0	Mixed, mainly mature.
	<i>Pennisetum ciliare</i>	0.21	0.34	0.27	0.61	0.36	0.58	8.0	Mixed, mainly mature.
	<i>Setaria gerrardi</i>	0.33	0.32	0.20	1.33	0.05	0.64	2.4	Mixed, practically all mature.
	<i>Ischaemum glauco-</i> <i>stachyum</i>	0.21	0.32	0.10	0.63	0.04	0.18	2.0	Mixed, practically all mature.
	<i>Amphilophis pertusa</i>	0.36	0.42	0.18	1.34	0.06	0.35	2.4	Mixed, practically all mature.
	<i>Digitaria</i> species.....	0.32	0.35	0.14	1.23	0.06	0.38	3.5	Mixed, practically all mature.
	<i>Panicum laevifolium</i>	0.21	0.38	0.33	2.06	0.07	0.62	5.5	Mixed, mainly mature.
Mimosa Park, Potchef. stroom	<i>Cynodon dactylon</i>	0.14	0.32	0.15	1.09	0.18	0.21	4.5	Mixed.
	<i>Aristida barbicollis</i>	0.23	0.42	0.17	0.92	0.03	0.23	4.4	Mixed, mainly green.
	<i>Eragrostis atherstonei</i> ...	0.14	0.38	0.18	0.73	0.03	0.22	3.1	Mixed, mainly green.
	<i>Themeda triandra</i>	0.11	0.36	0.25	0.74	0.08	0.18	2.1	Mixed, mainly mature.
Zulu National Training Inst., Nongoma	<i>Hypparrhenia hirta</i>	0.11	0.21	0.25	0.48	0.06	0.23	3.5	Mixed, practically all mature.
	<i>Panicum maximum</i>	0.21	0.46	0.43	0.95	1.00	0.70	6.9	Mixed.
	<i>Rhynchosyrrum roseum</i> ...	0.16	0.55	0.42	0.90	0.08	0.36	3.7	Mixed, mainly mature
	<i>Chloris variegata</i>	0.25	0.45	0.27	0.82	0.94	1.44	6.8	Mixed.
Koppieskraal, Kokstad.	<i>Eragrostis plana</i>	0.23	0.29	0.10	0.87	0.21	0.26	3.9	Mixed, mainly mature.
	<i>Themeda triandra</i>	0.18	0.28	0.13	0.75	0.14	0.16	3.9	Mixed.
	<i>Hypparrhenia hirta</i>	0.25	0.29	0.32	0.63	0.07	0.17	2.5	Mixed.

TABLE 4—(continued).

2

Area.	Species.	P ₂ O ₅ .	CaO.	MgO.	K ₂ O.	Na ₂ O.	Cl.	Protein.	Nature of Grass.
Lots Nos. 45/48, Entumeni, Eshowe	<i>Cymbopogon validus</i>	0.11	0.25	0.18	1.25	0.15	0.80	2.7	Mixed.
	<i>Eragrostis nebulosa</i>	0.16	0.24	0.05	0.70	0.10	0.34	2.4	Mixed, mainly mature.
	<i>Digitaria</i> species.....	0.16	0.24	0.10	0.83	0.32	0.87	1.9	Mixed, mainly mature.
	<i>Aristida junceiformis</i>	0.09	0.20	0.07	0.33	0.06	0.11	3.1	Mixed, mainly mature.
	<i>Hyparrhenia hirta</i>	0.11	0.34	0.13	0.55	0.04	0.10	2.5	Mixed, practically all mature.
Mimosa, Umtata.....	<i>Hyparrhenia hirta</i>	0.11	0.35	0.17	0.74	0.05	0.22	2.3	Mixed, mainly mature.
	<i>Digitaria</i> species.....	0.23	0.27	0.12	1.82	0.46	0.90	3.1	Mixed, mainly mature.
	<i>Cynodon dactylon</i>	0.43	0.27	0.07	1.72	0.36	0.60	7.3	Mixed.
	<i>Sporobolus indicus</i>	0.16	0.20	0.10	1.04	0.26	0.42	2.2	Mixed, mainly mature.
	<i>Themeda triandra</i>	0.14	0.43	0.13	0.61	0.04	0.15	2.9	Mixed.
Mt. Hupeleya, Queens-town	<i>Digitaria</i> species.....	0.34	0.50	0.20	2.25	0.32	0.58	8.3	Green.
	<i>Aristida barbicollis</i>	0.23	0.39	0.15	0.56	0.04	0.07	4.9	Mixed, mainly mature.
	<i>Eragrostis micrantha</i>	0.32	0.34	0.10	0.99	0.04	0.25	7.0	Green.
	<i>Cynodon dactylon</i>	0.41	0.52	0.22	2.01	0.19	0.33	8.5	Mixed.
Woodlands, Bathurst..	<i>Aristida junceiformis</i>	0.18	0.21	0.12	0.41	0.14	0.29	4.2	Mixed, mainly mature.
	<i>Eragrostis plana</i>	0.14	0.21	0.10	0.41	0.12	0.12	3.1	Mixed.
	<i>Themeda triandra</i>	0.23	0.31	0.13	0.55	0.14	0.17	5.0	Mixed, mainly mature.
Allandale, Middelburg, C.P.	<i>Pentzia incana</i> (Sensii Lat.)	0.30	0.59	0.16	1.55	0.55	0.18	8.6	Shrub.
	<i>Chrysocoma tenuifolia</i> ... <i>Eragrostis atherodes</i>	0.27 0.71	0.80 0.36	0.25 0.12	1.74 0.86	0.62 0.18	0.19 0.21	7.0 4.9	Shrub. Mixed, mainly green.
Kingston, Albany.....	<i>Panicum maximum</i>	0.94	0.38	0.28	1.11	0.47	0.52	5.2	Mixed, mainly mature.
	<i>Chrysocoma tenuifolia</i> ...	0.66	0.70	0.30	2.4	0.78	0.78	9.2	Shrub.
	<i>Pentzia incana</i> (Sensii Lat.)	0.71	1.12	0.50	2.06	1.21	0.70	10.8	Green, shrub.
	<i>Cussonia</i> species.....	0.76	1.20	0.56	3.27	0.51	0.36	13.5	Green, shrub.
	<i>Portulacaria affra</i>	0.39	0.98	1.02	3.24	2.58	0.85	7.5	Green, shrub.
	<i>Themeda triandra</i>	0.39	0.32	0.10	0.75	0.03	0.17	3.3	Mixed, mainly mature.

TABLE 4—(continued).

Area.	Species.	P ₂ O ₅ .	CaO.	MgO.	K ₂ O.	Na ₂ O.	Cl.	Protein.	Nature of Grass.
Greefputs, Barkly West	<i>Rhynchelytrum roseum</i> ...	0.32	0.29	0.23	1.09	0.32	0.29	6.0	Mixed.
	<i>Themeda triandra</i>	0.09	0.31	0.15	0.61	0.11	0.08	3.7	Mixed, mainly mature.
	<i>Eragrostis major</i>	0.11	0.22	0.83	0.53	0.07	0.08	3.3	Mixed, practically mature.
	<i>Digitaria</i> species.....	0.14	0.29	0.12	1.40	0.15	0.22	5.6	Mixed, mainly green.
	<i>Pogonarthria falcatula</i>	0.23	0.24	0.12	1.11	0.23	0.25	6.3	Mixed, mainly mature.
	<i>Eragrostis lehmanniana</i> ...	0.11	0.28	0.08	0.50	0.07	0.09	4.2	Mixed, practically all mature.
	<i>Schmidtia bulbosa</i>	0.11	0.27	0.11	0.53	0.08	0.11	5.7	Mixed, mainly mature.
Glen Shields, Bloemfontein	<i>Aristida uniplumis</i>	0.11	0.21	0.07	0.51	0.03	0.05	4.3	Mature.
	<i>Themeda triandra</i>	0.23	0.27	0.28	0.86	0.04	0.15	3.5	Mixed, mainly mature.
	<i>Chrysocoma tenuifolia</i> ...	0.73	0.85	0.56	2.5	—	0.43	13.5	Shrubs.
	<i>Eragrostis curvula</i>	0.32	0.34	0.27	0.79	—	0.14	5.0	Mixed.
	<i>Chloris virgata</i>	0.30	0.32	0.15	1.79	0.32	0.41	3.3	Mixed, practically all mature.
	<i>Digitaria</i> species.....	0.39	0.28	—	1.55	0.35	0.52	3.6	Mixed.
	<i>Eragrostis biflora</i>	0.30	0.38	0.17	1.31	0.27	0.40	3.0	Mixed.
Naseby Thorns, Kroonstad	<i>Themeda triandra</i>	0.30	0.31	0.08	0.81	0.06	0.16	2.5	Mixed, mainly mature.
Arnoedsvalke, Vryburg	<i>Fingerhuthia africana</i> ...	0.16	0.14	0.17	1.00	0.11	0.07	4.8	Mixed.
	<i>Digitaria</i> species.....	0.11	0.56	0.17	1.04	0.08	0.17	3.3	Mixed, practically all mature.
	<i>Eragrostis superba</i>	0.14	0.41	0.83	0.77	0.06	0.09	3.7	Mixed, mainly mature.
	<i>Rhynchelytrum roseum</i> ...	0.18	0.55	0.32	1.11	0.13	0.15	5.0	Mixed, mainly mature.
	<i>Chrysoporton</i> species.....	0.11	0.53	0.10	0.70	0.06	0.18	3.8	Mixed, practically all mature.
	<i>Elenine indica</i>	0.18	0.57	0.45	1.19	0.20	0.20	6.8	Mixed, practically all mature.
	<i>Aristida junceaformis</i>	0.16	0.18	—	0.35	0.01	0.05	4.6	Mixed, practically all mature.
Leliefontein No. 25, Ermelo	<i>Themeda triandra</i>	0.11	0.36	—	0.70	0.10	0.19	2.4	Mixed.
	<i>Monocymbium cerasiforme</i>	0.14	0.36	0.13	0.45	0.03	0.08	2.0	Mixed, mainly mature.
	<i>Urochloa pallulans</i>	0.41	0.62	0.33	3.08	0.49	1.01	6.9	Mixed, mainly mature.
	<i>Digitaria</i> species.....	0.27	0.66	0.28	1.94	0.41	0.46	4.1	Mixed, mainly mature.
	<i>Chloris virgata</i>	0.34	0.66	—	2.88	0.50	1.07	4.6	Mature.
	<i>Pennisetum ciliare</i>	0.16	0.35	—	1.61	0.24	0.35	4.5	Mixed, practically all mature.
	<i>Panicum maximum</i>	0.53	0.63	—	2.54	0.47	0.50	5.7	Mixed, practically all mature.

TABLE 4—(continued).

Area.	Species.	P ₂ O ₅ .	CaO.	MgO.	K ₂ O.	Na ₂ O.	Cl.	Protein.	Nature of Grass.
Kaalplaats, Marico.....	<i>Themeda triandra</i>	0.11	0.34	0.17	0.54	0.04	0.14	2.4	Mixed, mainly mature.
	<i>Cymbopogon plarinodis</i> ...	0.07	0.35	0.13	0.57	0.10	0.20	2.4	Mixed, practically all mature.
	<i>Digitaria</i> species.....	0.07	0.58	0.37	0.62	0.06	0.21	2.4	Mature.
	<i>Trachypogon polymorphus</i>	0.07	0.22	0.12	0.45	0.03	0.13	1.6	Mature.
	<i>Eragrostis biflora</i>	0.07	0.29	0.10	0.31	0.04	0.06	2.4	Mixed, practically all mature.
	<i>Eragrostis</i> species.....	0.07	0.38	0.12	0.38	0.04	0.12	4.7	Mixed, practically all mature.
	<i>Trichopteryx simplex</i>	0.07	0.20	0.08	0.36	0.07	0.11	1.8	Mature.
MINERAL SURVEY III, OCTOBER, 1931.									
Barberton.....	<i>Themeda triandra</i>	0.32	0.31	0.17	0.80	0.01	0.16	3.6	Mixed, mainly mature.
	<i>Sporobolus indicus</i>	0.34	0.28	0.15	0.92	0.04	0.20	3.6	Mixed, mainly green.
Kaalplaats.....	<i>Trichopteryx simplex</i>	0.07	0.20	0.10	0.10	0.01	0.04	1.7	Mature.
	<i>Themeda triandra</i>	0.09	0.27	0.13	0.25	0.03	0.07	2.5	Old, mature.
Gemsbokpan, Mafeking	<i>Themeda triandra</i>	0.07	0.20	0.14	0.59	0.03	0.16	1.6	Old, mature.
	<i>Aristida</i> species.....	0.07	0.24	0.12	0.53	0.03	0.07	3.7	Old, mature.
	<i>Digitaria</i> species.....	0.07	0.27	0.23	0.86	0.03	0.16	2.3	Mixed, practically all mature.
Derby 56, Piet Retief..	<i>Eragrostis curvula</i>	0.21	0.35	0.13	0.42	0.01	0.13	3.6	Mixed, practically all mature.
	<i>Themeda triandra</i>	0.14	0.34	0.12	0.42	0.03	0.13	1.7	Mixed, practically all mature.
Wildebeestfontein, Petersburg.....	<i>Elgonurus argenteus</i>	0.11	0.39	0.19	0.40	0.01	0.11	3.5	Mixed.
	<i>Aristida congesta</i>	0.11	0.20	0.07	0.25	0.01	0.05	2.5	Mixed, mainly mature.
	<i>Themeda triandra</i>	0.14	0.28	0.12	0.48	0.01	0.13	2.0	Mixed, mainly mature.
	<i>Heteropogon contortus</i> ...	0.09	0.24	0.15	0.36	0.01	0.11	2.7	Mixed, mainly mature.
Mimosa Park, Potchef- stroom	<i>Aristida</i> species.....	0.05	0.18	0.07	0.15	0.01	0.02	1.5	Mixed, practically all mature.
	<i>Themeda triandra</i>	0.07	0.17	0.07	0.31	0.01	0.06	2.2	Old, mature.
	<i>Cynodon dactylon</i>	0.09	0.34	0.11	0.27	0.01	0.05	3.6	Mixed, mainly mature.
Lets Nos. 45/48, Entu- meni, Eshowe	<i>Hypparrhenia hirta</i>	0.11	0.24	0.15	0.54	0.07	0.28	3.0	Mixed, mainly mature.
	<i>Aristida junceaformis</i>	0.14	0.21	0.06	0.38	0.03	0.12	4.1	Mixed, mainly green.
	<i>Eragrostis nebulosa</i>	0.16	0.28	0.15	0.85	0.36	0.70	3.5	Mixed, practically all mature.
Rockdale, Kingwilliams- town	<i>Sporobolus indicus</i>	0.37	0.32	0.18	1.27	0.09	0.44	8.0	Green.
	<i>Themeda triandra</i>	0.37	0.38	0.20	1.28	0.14	0.48	10.0	Green.

TABLE 4—(continued).

Area.	Species.	P ₂ O ₅ .	CaO.	MgO.	K ₂ O.	Na ₂ O.	Cl.	Protein.	Nature of Grass.
Koppieskraal, Kokstad.	<i>Eragrostis plana</i>	0.32	0.25	0.16	0.73	0.23	0.32	6.9	Mixed.
	<i>Themeda triandra</i>	0.16	0.38	0.26	0.59	0.03	0.19	3.7	Mixed, mainly green.
	<i>Cymbopogon</i> species....	0.21	0.27	0.32	0.57	0.01	0.17	3.2	Mixed, practically all mature.
Mimosa Farm, Umtata	<i>Eragrostis curvula</i>	0.21	0.29	0.08	0.57	0.04	0.24	4.1	Mixed.
	<i>Themeda triandra</i>	0.18	0.45	0.11	0.65	0.04	0.17	3.4	Mixed.
Mt. Hupeley, Queens- town	<i>Aristida barbicollis</i>	0.16	0.36	0.07	0.79	0.03	0.04	4.9	Mixed, mainly mature.
	<i>Eragrostis micrantha</i> ...	0.25	0.41	0.09	1.03	0.03	0.24	7.1	Mixed, mainly green.
Prinestone, Bedford...	<i>Diplachne fusca</i>	0.27	0.25	0.12	0.80	0.34	0.73	5.1	Mixed, mainly green.
	<i>Cynodon dactylon</i>	0.73	0.41	0.17	1.61	0.46	0.53	11.0	Mixed.
	<i>Themeda triandra</i>	0.30	0.46	0.16	0.91	0.12	0.23	6.6	Mixed.
	<i>Chrysocoma tenuifolia</i> ...	0.37	1.71	0.43	2.31	0.09	0.85	8.5	Shrub.
	<i>Medicago denticulata</i> ...	0.87	1.61	0.67	4.16	0.29	1.13	8.5	Shrub.
Lombardspost, Bathurst	<i>Setaria</i> species.....	0.32	0.28	0.06	0.98	0.38	0.48	7.3	Green.
	<i>Eragrostis brizoides</i>	0.21	0.49	0.09	0.76	0.12	0.20	4.6	Green.
Allandale, Middelburg, C.P.	<i>Eragrostis atherstonel</i> ...	0.27	0.32	0.11	0.44	0.11	0.11	5.7	Mixed, mainly mature.
	<i>Pennisetum</i>	0.25	0.90	0.08	1.72	0.05	0.12	6.1	Shrub.
	<i>Chrysocoma tenuifolia</i> ...	0.23	0.73	0.40	1.84	0.16	0.20	6.9	Shrub.
Greefputs, Barkly West	<i>Heteropogon contortus</i> ...	0.05	0.24	0.27	0.52	0.01	0.06	2.5	Old, mature.
	<i>Aristida uniplanis</i>	0.07	0.22	0.58	0.33	0.01	0.04	2.7	Old, mature.
	<i>Digitaria</i> species.....	0.07	0.42	0.24	0.53	0.01	0.05	3.6	Old, mature.
	<i>Schmidtia bulbosa</i>	0.07	0.27	0.11	0.61	0.01	0.11	3.8	Old, mature.
	<i>Themeda triandra</i>	0.05	0.20	0.08	0.46	0.01	0.08	1.7	Old, mature.
Glen Shields, Bloem- fontein	<i>Themeda triandra</i>	0.09	0.24	0.12	0.50	0.03	0.08	2.8	Mixed, practically all mature.
	<i>Chrysocoma tenuifolia</i> ...	0.71	1.36	0.61	1.08	0.44	0.43	8.6	Shrub.
Naseby Thorns, Kroon- stad	<i>Themeda triandra</i>	0.12	0.24	0.16	0.44	0.03	0.04	2.2	Mixed, mainly mature.
	<i>Digitaria</i> species.....	0.23	0.56	0.21	0.53	0.04	0.11	4.4	Mature.
	<i>Aristida</i> species.....	0.09	0.22	0.07	0.16	0.03	0.02	2.5	Mature.
	<i>Eragrostis</i> species.....	0.23	0.27	0.16	0.53	0.03	0.17	4.5	Mixed, mainly mature.

Discussion.

Table 4 is very interesting as the composition of the various species collected in one area have been subject to the same conditions of climate and soil fertility and are, therefore, more strictly comparable. The difference in chemical composition between a number of species from the same area is therefore due chiefly to natural differences among the species themselves, such as more leaf formation, high in phosphorus in one species, e.g. *Panicum maximum*, *Themeda triandra*, etc., as against excess stalk formation of poor analyses in the other, e.g. *Aristida congesta*. Furthermore, grasses show differences in the rate of growth, some being early and others late varieties. Some are better drought resisters than others or show better growth on a poor soil. The effects of these combined factors, grouped under characteristics of species, on the chemical composition of grasses must be borne in mind when considering Table 4.

Panicum maximum was collected in four different areas in May, 1931. In three cases its analyses were quite the best and, in the fourth its phosphorus content was still the highest, the rest of its composition excellent, but there was little to choose between it and some of the other species. Apparently *Panicum maximum*—"buffel gras"—produced excellent winter grazing in 1931.

Cynodon dactylon—"kweek"—although its chemical composition is definitely not on a par with the species just mentioned, also shows quite fair analyses and good winter grazing. *Themeda triandra*—"rooi gras"—one of the commonest grasses and collected from about twenty areas, was low to medium in phosphorus in May, 1931, and leaves much to be desired as winter grazing. Other species could be selected, but it will be more interesting to compare the chemical compositions at a later date when the analyses at other seasons are available.

5. INORGANIC PHOSPHORUS IN THE BLOOD.

The samples of precipitated blood sent in from the field as stated earlier in this publication, were analysed for inorganic phosphorus on arrival. The results of the analyses are given in Table 5.

Discussion.

The most important factor in Table 5, apart from the values for phosphorus, is undoubtedly the period of hydrolysis. Provisionally the increase due to hydrolysis will be considered on the following basis. Values for inorganic phosphorus after 24 hours hydrolysis will be regarded as 10 per cent. above the true value, after two days 20 per cent., and beyond that time more than 20 per cent. Data will be presented on this phase of the work in the near future. It may only be added that judging from the figures obtained so far the difficulty of deriving at true values owing to the hydrolytic factor, will be completely removed.

TABLE 5.

INORGANIC PHOSPHORUS GIVEN IN MG. per 100 c.c. BLOOD.

District.	Farm.	Mineral Survey.	Inorganic Phosphorus Average of 10 Animals.	Description of Animals.	Period of Hydrolysis (Days).
(1) Middelburg, Transvaal.....	Rodepoort No. 152..... Aberdeen No. 291..... Rodepoort No. 8..... Mootwater No. 129..... Rodepoort No. 8.....	1 (May, '30) 2 (May, '31) 3 (Oct., '31) 4 (Jan., '32) 5 (April, '32)	7·0 5·1 3·1 5·4 3·4	Young oxen and heifers..... Oxen, 8-10 years..... Young heifers..... Dry cows..... Lactating cows.....	1 2 1 2 1
(2) Krugersdorp, Transvaal.....	Hartebeestfontein No. 51..... Hartebeestfontein No. 51..... " " " " " "	1 2 3 4 5	4·1 — 5·3 4·0 4·2	Oxen..... — Oxen, 7-8 years..... Oxen, 4-6 years..... Oxen, 4-6 years.....	2 — 1 1 1
(3) Barberton, Transvaal.....	Brooklyn..... — Brooklyn..... — —	1 2 3 4 5	5·1 — 5·7 — —	Oxen..... — Dry cows, oxen and heifers..... — —	5 — 3 — —
(4) Lydenburg, Transvaal.....	Frischgewaagd No. 82..... De Grootboom No. 214..... " " " " " " —	1 2 3 4 5	5·1 6·0 6·7 — —	Oxen..... Heifers and oxen, 3 years..... Heifers, 2 years..... — —	2 4 2 — —

TABLE 5—(continued).

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District.	Farm.	Mincral Survey.	Inorganic Phosphorus Average of 10 Animals.	Description of Animals.	Period of Hydrolysis (Days).
(5) Marico, Transvaal.....	Niet Verdient No. 196.....	1	4.2	Lactating cows, 3½ years.....	3
	Kaalplaats No. 97.....	2	3.8	Dry ewes.....	2
	" ".....	3	6.6	Full mouth ewes.....	3
	" ".....	4	6.0	Ewes.....	3
(6) Zoutpansberg, Transvaal.....	" ".....	5	5.9	Ewes.....	4
	Government Ranching Station..	1	—	—	—
	" ".....	2	4.4	Lactating cows, 4-8 years.....	4
	" ".....	3	4.8	Dry and lactating cows.....	1
(7) Potgietersrust, Transvaal.....	" ".....	4	5.4	Dry cows.....	1
	" ".....	5	4.6	—	1
	Hebron No. 715.....	1	3.7	Pregnant heifers, 2½ years.....	1
	Mooigelegen No. 635.....	2	4.3	Oxen, 4 years, fair condition....	3
(8) Piet Retief, Transvaal.....	Mooigelegen No. 635.....	3	3.1	—	—
	—	4	1.1	Lactating cows.....	1
	—	5	—	—	—
	Derby No. 56.....	1	—	—	—
(9) Pietersburg, Transvaal.....	" ".....	2	4.6	Dry cows and heifers, 4 years...	3
	" ".....	3	3.6	Dry cows.....	1
	" ".....	4	4.5	Dry cows.....	1
	" ".....	5	3.1	Dry cows.....	1
(9) Pietersburg, Transvaal.....	Wildebeestfontein No. 89.....	1	4.7	Oxen, 5 years.....	5
	" ".....	2	5.0	Dry cows and heifers, 4 years...	1
	" ".....	3	3.2	Dry cows.....	1
	" ".....	4	3.9	Dry cows.....	1
(9) Pietersburg, Transvaal.....	—	5	—	—	—

TABLE 5—(continued).

District.	Farm.	Mineral Survey.	Inorganic Phosphorus Average of 10 Animals.	Description of Animals.	Period of Hydrolysis (Days).
(10) Potchefstroom, Transvaal.....	Rietfontein No. 503.....	1	4.6	Dry cows.....	5
	Mimosa Park.....	2	3.2	Lactating cows, 6 years.....	3
	"	3	3.4	Dry and lactating cows.....	2
	"	4	3.6	Dry and lactating cows.....	2
(11) Ernelo, Transvaal.....	"	5	2.4	Dry and lactating cows.....	3
	—	—	—	—	—
	Leliefontein.....	1	5.9	Yearling heifers.....	4
	"	2	3.7	2 year heifers.....	1
(12) Ixopo, Natal.....	"	3	2.6	Lactating cows.....	2
	—	5	—	—	—
	Stanton.....	1	3.2	Dry cows, 9 years.....	2
	"	2	4.8	Oxen, 8 years.....	3
(13) Vryheid, Natal.....	"	3	5.5	Dry cows.....	4
	"	4	4.4	2 year old heifers.....	3
	—	5	—	—	—
	Uitvlugt.....	1	4.8	Lactating cows feeding on mealie stalks	3
(14) Port Shepstone, Natal.....	Bergendal.....	2	2.2	Dry cows, 6 years.....	4
	"	3	3.9	Oxen, 3 years.....	2
	"	4	3.3	Dry cows.....	3
	"	5	4.0	Dry cows.....	5
(14) Port Shepstone, Natal.....	—	—	—	—	—
	Melbourne.....	1	3.4	Lactating cows.....	4
	"	2	3.2	Lactating cows.....	3
	"	3	3.7	Lactating cows.....	3
(14) Port Shepstone, Natal.....	"	4	3.1	Lactating cows.....	3
	"	5	—	—	—

TABLE 5—(continued).

District.	Farm.	Mineral Survey.	Inorganic Phosphorus Average of 10 Animals.	Description of Animals.	Period of Hydrolysis (Days).
(15) Eshowe, Natal.....	Arcadia..... Lots Nos. 45/48, Entumeni.... " " " " " " " " "	1 2 3 4 5	3·9 4·2 5·0 7·2 5·0	Dry cows, 4-5 years..... Dry cows, 4 years..... Heifers, 3 years..... Heifers, 3 years..... Dry cows and heifers.....	3 4 3 3 4
(16) Greytown, Natal.....	Voorkeur..... Area No. 24, Umvoti..... " " Voorkeur.....	1 2 3 4 5	4·1 4·9 5·4 — 3·0	Lactating cows..... Dry and lactating cows, 5 years. Dry cows..... — Lactating cows.....	5 3 3 — 3
(17) Kingwilliamstown, Cape Province.	Gleniffer..... Rockdale..... " " " " " "	1 2 3 4 5	5·1 3·5 3·3 3·5 3·6	Oxen, 5 years..... Lactating cows..... Dry and lactating cows..... — Dry cows and heifers.....	3 4 3 — 6
(18) Kokstad, Cape Province.....	Koppieskraal..... " " " "	1 2 3 4 5	4·8 4·2 5·3 5·6 5·1	Working oxen..... Pregnant heifers..... Yearling heifers..... Oxen, heifers and dry cows..... Working oxen, and heifers.....	4 4 3 4 4
(19) Queenstown, Cape Province.....	Endwell..... Mount Hupeley..... " " " " "—	1 2 3 4 5	5·7 5·0 5·9 3·2 —	Lactating cows..... Dry cows..... Yearling heifers..... Lactating cows..... —	3 3 2 2 —

TABLE 5—(continued).

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District.	Farm.	Mineral Survey.	Inorganic Phosphorus Average of 10 Animals.	Description of Animals.	Period of Hydrolysis (Days).
(20) Umtata, Cape Province.....	Zwartfontein.....	1	5.2	Oxen, 7 years old.....	3
	Min.osa Farm.....	2	4.5	Dry cows, 7 years.....	3
	" ".....	3	3.5	Dry cows, 5 years.....	3
	" ".....	4	4.6	Dry cows.....	3
(21) Bedford, Cape Province.....	" ".....	5	5.0	Dry cows.....	3
	Primestone.....	1	4.1	Young bulls, 2 years.....	4
	" ".....	2	6.2	Yearling heifers.....	2
	" ".....	3	5.2	Heifers, 2 years.....	4
(22) De Aar, Cape Province.....	" ".....	4	7.6	Heifers and oxen.....	4
	" ".....	5	6.0	Oxen.....	4
	—	1	—	—	—
	—	2	—	—	—
(23) Bathurst, Cape Province.....	Rustfontein.....	3	4.6	Dry cows.....	2
	" ".....	4	5.3	Heifers, oxen and dry cows.....	2
	" ".....	5	4.0	Dry cows and oxen.....	2
	Woodlands.....	1	4.3	Oxen, 4 years old.....	3
(24) Albany, Cape Province.....	" ".....	2	4.2	Tollies, 3 years old.....	5
	Lombardspost.....	3	5.7	Heifers, 2 years.....	4
	" ".....	4	6.3	Dry cows.....	3
	" ".....	5	3.8	Dry cows.....	3
(24) Albany, Cape Province.....	Penrock.....	1	6.4	Dry cows and heifers.....	3
	Kingston.....	2	5.9	Lactating cows.....	4
	" ".....	3	4.9	Lactating cows, 3 years.....	4
	" ".....	4	7.8	Heifers.....	3
(24) Albany, Cape Province.....	" ".....	5	5.8	Dry cows.....	4
	" ".....	5	5.8	Dry cows.....	4

TABLE 5—(continued).

District.	Farm.	Mineral Survey.	Inorganic Phosphorus Average of 10 Animals.	Description of Animals.	Period of Hydrolysis (Days).
(25) Middelburg, Cape Province.....	—				
	Allandale.....	1	—	Lactating cows.....	—
	"	2	6.1	Dry and lactating cows.....	3
	"	3	4.6	Lactating cows.....	4
(26) Barkly West, Cape Province.....	—				
	Greefputs.....	1	—	Dry cows.....	—
	"	2	4.6	Dry cows and heifers.....	4
	"	3	3.4	Dry cows.....	2
(27) Port Elizabeth, Cape Province....	—				
	Craddock Place.....	1	—	Oxen, very good condition.....	—
	"	2	9.6	Oxen, 6 years.....	5
	"	3	6.3	Oxen.....	3
(28) Lusikisiki, Cape Province.....	—				
	"	4	6.6	Oxen and cows.....	3
	"	5	8.0		4
	Xura Area.....	1	4.3	Lactating cows.....	6
(29) Pinetown, Natal.....	—				
	"	2	—	Oxen, lactating cows and heifers	—
	"	3	4.4	Heifers, oxen and lactating cows	4
	"	4	5.0		6
(29) Pinetown, Natal.....	—				
	Marianhill.....	1	3.5	Oxen.....	5
	Zeekoegat.....	2	4.8	Oxen.....	5
	Marianhill.....	3	5.3	Oxen.....	4
(29) Pinetown, Natal.....	—				
	"	4	5.5	Oxen.....	3
	"	5	—		—

TABLE 5—(continued).

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District.	Farm.	Mineral Survey.	Inorganic Phosphorus Average of 10 Animals.	Description of Animals.	Period of Hydrolysis (Days).
(30) Dundee, Natal.....	—	1	—	—	—
	Halifax.....	2	—	—	—
	Moss Side.....	3	5.3	Oxen.....	2
	" ".....	4	3.2	Dry cows and oxen.....	2
	" ".....	5	4.1	Working oxen and dry cows....	3
(31) Bloemfontein, Orange Free State..	Bishop's Glen.....	1	4.1	Lactating cows.....	3
	Glen Shields.....	2	4.8	Dry cows.....	2
	" ".....	3	3.7	Dry cows.....	2
	" ".....	4	5.3	Dry cows.....	2
	" ".....	5	4.6	Dry cows.....	2
(32) Kroonstad, Orange Free State....	—	1	—	—	—
	Naseby Thorns.....	2	7.5	Dry cows and heifers.....	3
	" ".....	3	7.8	Young bulls and heifers.....	2
	" ".....	4	6.1	Dry cows and young bulls.....	2
	" ".....	5	5.7	Dry cows.....	2
(33) Bethlehem, Orange Free State....	The Outlook.....	1	3.8	Working oxen, poor condition, 4 years	2
	—	2	—	—	—
	The Outlook.....	3	4.2	Oxen, 3 years.....	2
	" ".....	4	5.5	Lactating cows.....	3
	" ".....	5	3.8	Dry and lactating cows.....	2

TABLE 5—(continued).

District.	Farm.	Mineral Survey.	Inorganic Phosphorus Average of 10 Animals.	Description of Animals.	Period of Hydrolysis (Days).
(34) Impendhle, Natal..... Camperdown, Natal..... Richmond, Natal..... Pietermaritzburg, Natal..... —	Vandusz.....	1	3.3	Dry cows.....	2
	Mountain View.....	2	5.4	Oxen.....	2
	Commissiedrift.....	3	6.6	Oxen.....	2
	Braeburn.....	4	5.0	Oxen.....	2
(35) Mafeking, Cape Province.....	—	5	—	—	—
	Gembokpan.....	1	—	—	—
	"	2	—	—	—
	"	3	3.7	Lactating cows and ewes.....	6
(36) Marico, Transvaal.....	"	4	4.2	Lactating cows and ewes.....	4
	"	5	6.2	Ewes.....	6
	—	1	—	—	—
	—	2	—	—	—
(37) Zwartruggens, Transvaal.....	—	3	4.6	—	3
	—	4	3.5	—	2
	—	5	3.8	—	—
	—	—	—	—	—
(37) Zwartruggens, Transvaal.....	—	1	—	—	—
	—	2	—	—	—
	—	3	4.2	—	—
	—	4	6.7	—	—
(37) Zwartruggens, Transvaal.....	—	5	3.9	—	—
	—	—	—	—	—
	—	—	—	—	—
	—	—	—	—	—

For the present, therefore, a consideration of the results of Table 5 with the variations in periods of hydrolysis from 1-6 days and for blood obtained from cattle of different ages will be followed on general lines only. The inorganic phosphorus in the blood of young stock is always definitely higher than that of older stock. Heifers and oxen 2 years old, on phosphorus sufficient pasture, will probably average round about 5.3 mgm. per 100 c.c. blood, according to the values given by Malan, Green and Du Toit (1927), while mature cattle generally would show approximately 3.8 mgm. under the same conditions. The blood of lactating cows is always lower—about 3.2 mgm., while the inorganic phosphorus in the blood of dry cows would be above 3.2 under conditions of phosphorus sufficiency. For the purpose of comparison, these figures may be taken as standards and allowance made for hydrolysis as suggested above.

A glance at Table 5 reveals that a number of areas from which blood was collected undoubtedly suffered from a marked degree of aphosphorosis. Perhaps most outstanding and obvious are areas 7, 8, 10, 11, 12, 13, 14, 17, etc., i.e. where figures round about 3 have been obtained in spite of hydrolysis which would normally have increased the figure after 2 or more days to considerably beyond that value. A more detailed study of the table is interesting. In area 1 the same farm, viz., Roodepoort, No. 8, was selected for only two of the five surveys by the field officer. In October, 1931, young heifers showed a figure of 3.1 mgm. inorganic phosphorus after 24 hours' hydrolysis. This figure is undoubtedly indicative of marked aphosphorosis, while 3.4 mgm. obtained for lactating cows in April, 1932—i.e. the fifth survey—after 24 hours hydrolysis does not lie far below the normal value. These two sets of figures incidentally bring out another advantage of blood analysis. The donors may suffer from varying degrees of acuteness of phosphorus deficiency, depending on the nature and abundance of the pasture. For instance, it is more than likely that pasture in its early stages of growth even on a soil deficient in phosphorus contains enough phosphorus for the maintenance of animals and, may be, even for optimum growth. At all events, there is no difference in the phosphorus content of the blood of cattle grazing such pasture and in the blood of those receiving in addition a phosphorus supplement. Young succulent grass contains on a soil as deficient in phosphorus as that at Armoedsvlakte in the notoriously deficient Bechuanaland area, approximately .4 per cent. P_2O_5 , and as yet there is no reason for believing that such pasture does not provide enough phosphorus for animal requirements. However, the point merely is that various degrees of deficiency or even of sufficiency and deficiency may exist during the year or during seasons of poor feeding, such as winter, and on the other hand during seasons of abundant grazing of good quality, as is sometimes the case in summer. Under such conditions the same animals will obviously show a high figure for inorganic phosphorus during the period of abundance and one indicative of aphosphorosis when very poor grazing is available. The two values 3.1 for young heifers on Roodepoort in area 1 in October, 1931, and of 3.4 for lactating cows on the same farm in April, 1932, suggests such a condition in the area in question. Areas 2, 3 and 4 may be passed over without comment, but

No. 5 is interesting. On Kaalplaats dry ewes showed 3.8 mgm. inorganic phosphorus in their blood. The owner realizing the significance, began feeding bone meal, so that the subsequent values signify phosphorus sufficiency, as they are meant to.

The figures given for area 6 appear to be normal. Area 9 strongly savours of deficiency. Areas 7, 8, 10, 11, 12, 13, 14, 17 have already been dealt with. The values for areas 15 and 16 if considered in the light of the knowledge that hydrolysis of three days and more had taken place, i.e. the values are most probably more than 20 per cent. too high, all suggest low true values. The same applies to area 18. It is noteworthy how few high values are present throughout the table in spite of the not inconsiderable increase due to hydrolysis.

Area 27—Cradock Place, Port Elizabeth—shows the type of values that would be anticipated for blood from phosphorus-sufficient areas after several days hydrolysis. Need for more knowledge in the effect of hydrolysis on the inorganic fraction is apparent for a correct interpretation of the results obtained. The results in Table 5 are, therefore, presented provisionally, without further comment, until a more accurate estimate can be made of the increase in the blood of bovines of different classes and ages for varying periods of hydrolysis under the conditions of the surveys.

All the organic acid soluble phosphorus does not hydrolyze to inorganic phosphorus even if the trichloroacetic acid solution containing the blood be kept indefinitely. Actually, therefore, the rate of hydrolysis of organic acid soluble phosphorus must be determined and, therefore, incidentally, its quantity present in the blood of bovines under conditions of deficiency and sufficiency respectively, after various periods of hydrolysis.

The values for inorganic phosphorus presented in Table 5 are the average of ten. There is quite a fair amount of variation between individual results as will be evident from a study of the figures given in Table 6.

Discussion.

Any individual set of figures presented in Table 6 shows quite a fair amount of variation. It seems, however, that the results of the analyses of samples of blood from 10 animals are sufficient to provide an idea of the phosphorus sufficiency or deficiency of the herd. Both low and high values have been included in the table. It is well to remember where the variation in the values are greatest that in most cases the class and ages of the animals selected differ as indicated.

IV. CONCLUDING REMARKS.

1. CORRELATION OF RESULTS OBTAINED FOR PHOSPHORUS FROM SOIL, PASTURE AND BLOOD ANALYSES RESPECTIVELY.

The soil analyses presented in Table 1 leave no doubt that there is a marked deficiency of available phosphorus in most South African soils. As a matter of fact the table contains relatively few figures for available P_2O_5 above .005 per cent. A consideration of the phosphorus content of pastures presented in Tables 1, 3 and 4 conveys the same idea of a great lack of phosphorus in the grazing generally for

TABLE 6.

1

Area.	Blood Sample.	Survey : May, 1931.	Survey : October, 1931.	Survey : January, 1932.	Survey : April, 1932.
Wildebeestfontein, Pietersburg, Transvaal	1	5.3	4.2	5.8	—
	2	8.0	3.3	4.0	—
	3	5.8	2.4	3.7	—
	4	5.8	3.1	2.5	—
	5	3.9	3.4	5.1	—
	6	5.4	3.7	3.6	—
	7	5.0	2.7	3.5	—
	8	4.4	2.4	3.6	—
	9	6.1	4.1	3.5	—
	10	6.0	—	—	—
Mimosa Park, Potchefstroom, Transvaal	1	3.8	3.5	3.4	2.1
	2	3.2	4.5	4.2	2.5
	3	2.4	3.4	6.4	3.0
	4	3.3	3.4	1.8	2.0
	5	3.4	3.2	2.8	2.4
	6	2.4	3.1	4.3	3.1
	7	2.5	3.8	3.6	1.3
	8	2.4	2.9	3.7	3.0
	9	5.4	2.5	2.1	3.0
	10	2.5	3.9	3.8	1.9
Lehefontein, Ermelo, Transvaal	1	5.6	3.1	2.3	—
	2	—	3.6	2.9	—
	3	4.8	4.0	3.1	—
	4	5.8	3.4	3.2	—
	5	5.7	3.4	2.9	—
	6	5.1	4.2	2.5	—
	7	6.2	3.4	2.7	—
	8	7.8	3.5	2.4	—
	9	6.6	4.2	2.9	—
	10	5.5	3.9	2.5	—

TABLE 6—(continued).

2

Area.	Blood Sample.	Survey : May, 1931.	Survey : October, 1931.	Survey : January, 1932.	Survey : April, 1932.
Bergendal, Vryheid, Natal...	1	2.8	4.0	3.9	4.6
	2	2.7	3.2	4.4	3.8
	3	2.1	3.6	2.5	4.8
	4	2.8	4.1	3.1	4.0
	5	1.9	4.8	2.3	4.5
	6	2.1	5.2	—	3.2
	7	1.6	3.0	3.0	4.2
	8	1.7	3.6	3.7	4.1
	9	1.9	3.9	3.2	2.5
	10	2.2	—	—	4.2
Melbourne, Port Shepstone, Natal	1	2.9	4.1	4.0	3.5
	2	3.1	3.3	4.3	2.9
	3	3.5	2.3	4.0	3.9
	4	2.7	3.0	4.3	2.9
	5	2.3	2.7	3.7	3.4
	6	1.9	2.6	3.9	2.9
	7	4.6	2.4	3.0	2.4
	8	4.2	3.3	2.7	3.1
	9	3.6	4.0	2.9	3.7
	10	4.6	3.9	4.6	2.1
Rockdale, Kingwilliamstown, Cape Province	1	2.9	3.2	4.0	1.8
	2	3.6	2.9	2.5	3.1
	3	5.2	4.1	3.0	3.6
	4	3.3	3.3	—	5.0
	5	3.9	2.9	3.4	3.4
	6	5.2	3.5	3.1	3.1
	7	2.8	4.5	2.8	2.0
	8	3.3	2.1	5.0	3.8
	9	2.2	3.3	3.8	5.0
	10	2.6	—	4.1	5.6

TABLE 6—(continued).

Area.	Blood Sample.	Survey: May, 1931.	Survey: October, 1931.	Survey: January, 1932.	Survey: April, 1932.
Kingston, Albany, Cape Province	1	5.0	5.2	7.1	5.9
	2	6.6	5.0	7.5	5.7
	3	6.9	4.5	7.0	6.6
	4	6.0	4.8	7.5	6.0
	5	5.4	4.8	7.9	7.1
	6	6.0	3.3	8.0	5.0
	7	5.4	4.7	7.0	5.7
	8	6.3	5.3	9.0	5.2
	9	5.8	5.0	6.9	6.2
	10	5.1	5.8	9.9	4.9
Prinstone, Bedford, Cape Province	1	6.4	5.2	7.9	6.7
	2	6.4	5.4	7.7	5.7
	3	5.1	5.0	7.7	5.9
	4	5.8	5.6	7.8	5.9
	5	6.8	5.5	7.7	6.8
	6	7.0	3.5	7.6	5.6
	7	5.6	6.9	8.1	5.7
	8	4.8	5.0	8.0	6.7
	9	6.8	5.2	6.5	5.6
	10	6.8	4.4	6.5	5.6
Allandale, Middelburg, Cape Province	1	5.0	4.3	4.8	4.9
	2	6.1	6.3	6.6	5.4
	3	6.2	5.0	5.2	6.3
	4	5.0	5.0	5.9	4.5
	5	6.5	4.8	4.3	6.2
	6	6.0	5.7	5.3	4.7
	7	7.0	5.7	6.3	4.6
	8	5.8	5.7	7.1	4.6
	9	7.2	4.5	5.1	5.0
	10	5.8	5.0	5.4	4.3
Naseby Thorns, Koonstad, Orange Free State	1	7.3	6.2	7.5	5.7
	2	5.5	6.9	5.6	6.8
	3	5.5	9.2	5.4	5.9
	4	7.1	8.0	6.3	4.7
	5	7.2	5.9	6.0	6.3
	6	9.8	7.7	5.3	5.3
	7	8.7	6.6	7.3	5.2
	8	6.4	6.6	5.1	5.4
	9	7.1	9.2	5.8	—
	10	9.7	10.5	6.2	—

the periods mentioned. Here again there are only a few farms where the phosphorus content of the pasture for all three surveys is anything like a satisfactory figure. Blood analyses for inorganic phosphorus provide yet further proof of an almost generalized aphosphorosis in cattle grazing on the pasture analysed.

It would, therefore, seem necessary to find out to what extent the evidence for a phosphorus deficiency provided by soil or pasture or blood analyses is corroborated when the evidence of the remaining two sets of analyses are considered. In short, does a correlation exist between the values obtained for phosphorus by the three methods employed, viz., soil, pasture and blood analyses? Without reference to the tables it would seem impossible to obtain such a correlation between soil and pasture owing to the variation in the stage of growth of pasture in the areas to be compared. For instance, pasture consisting of mainly green vegetation growing on a soil very deficient in phosphorus will invariably be higher in its phosphorus content than that on a soil comparatively high in phosphorus but consisting of old mature grass. Areas 20 and 6 in Table 1 provide evidence for this statement, as is apparent from the following:—

Locality.	Survey.	Soil P.	Pasture P.	Description.
Area 20.....	3	·0004	·46	Green.
Area 6.....	2	·0431	·16	Mixed, practically all mature.
Area 1.....	3	·0003	·23	Mixed.
Area 7.....	1	·00035	·14	Mixed.

These examples could be multiplied from Table 1. At the same time it will be seen that if grasses be selected that are classified in the same category, e.g. mixed, or mixed mainly mature, etc., still no general correlation exists between soil and pasture figures. Here again it must be pointed out that the classification, although serving the very excellent purpose of indicating the state of the pasture, is arbitrary and cannot attempt to define the stage of growth of the pasture exactly. Old pasture in October nearly always contains young shoots and will, therefore, be classed as “mixed, practically all mature”, while nearly all pasture that has grown to maturity in late autumn will be classed in the same way. Obviously the stages of growth are different and it is not known definitely how the phosphorus content will vary with the age of the grass. The factors effecting growth are climatic mainly, and therefore beyond human control, so that theoretically it will be possible to have pasture in practically all stages of growth, and therefore of greatly varying phosphorus content on soils equally deficient or sufficient in phosphorus. In practice, however, seasonal growth limits the variations in stage of growth so that in winter, for instance, one would expect to find mainly mature grasses in most areas, but exceptions to such anticipations must not be regarded as extraordinary. Hence one would anticipate values for phosphorus under approximately similar climatic conditions and for the same season that are comparable in a general way. Comparisons of the available soil phosphorus with

the phosphorus content of pastures must take due regard of climatic conditions in order to have any value at all. Types of soil will obviously affect its water-holding capacity, which in its turn will affect the growth of the pasture, thereby causing a change in its phosphorus content. In a survey such as that described in this paper all types of soil are dealt with and rainfall varies between wide limits. For the pasture phosphorus to show a close correlation with the soil phosphorus under all these conditions means that the phosphorus deficiency problem is the main one under all conditions of pasture management and determines the phosphorus content of the pasture in spite of differences that may exist in the stage of growth. This contention is obviously incorrect, for, as already said, green grass will invariably show a higher phosphorus content on a soil poor in phosphorus than old mature grass on a soil comparatively rich in phosphorus. Comparisons of soil phosphorus have thus been made with the phosphorus in the pasture on general lines and under the three headings:—

- (a) low available phosphorus in soil—below .005 per cent.:
- (b) medium available P. in soil .005-.01 per cent.
- (c) high available P. in soil above .01 per cent.

The three equivalents in pasture have been taken to be—

- (a) low, below .30 per cent.
- (b) medium, .30-.45 per cent.
- (c) high, above .45 per cent.

The table of comparison is presented hereunder. It will be noticed that blood analyses have been included. These have been divided into low and high respectively, on the following lines:—

Class of Stock.	True value for Inorg. P.	I.P. after 24 Hours' Hydrolysis.	I.P. after 48 Hours' Hydrolysis.	I.P. after more than 2 Days' Hydrolysis.
Young Cattle.....	5.3	5.8	6.3	over 6.3
Mature Stock.....	3.8	4.2	4.6	over 4.6
Lactating Cows.....	3.2	3.5	3.8	over 3.8

All values in Table 5 less than those in the table above for the class of stock in question after the period of hydrolysis stated are designated "low", while the rest are "high".

Table 7.—L. M. H indicate low, medium, and high values respectively. The numbers under Locality refer to those given to the areas in Table 1.

Locality.	Survey.	Soil P.	Pasture P.	Blood P.
1.....	2, 3	L L	L L	H L
2.....	1, 3	L L	L L	L H
3.....	1, 3	H M	H L	H H
4.....	1, 3, 3	L H L	L M L	H L H
5.....	1, 3, 3	L L L	L L L	L L L
6.....	2, 3	H H	L H	L H
7.....	1, 2	L L	L L	L L
8.....	2, 3	L L	L L	L L
9.....	1, 2, 3	L L L	L L L	L L L
10.....	1, 2, 3	L L L	L L L	L L L
11.....	2	M	L	L
12.....	2	L	L	L
13.....	1, 2, 3	L L L	L L L	L L L
14.....	1, 2, 3.	L L L	L L L	L L L
15.....	1, 2, 3	L L L	L L L	L L L
16.....	1, 2, 3	L L L	L L L	L L L
17.....	1, 2, 3	L L L	L L M	L L H
18.....	2, 3	L L	L L	L L
20.....	1, 2, 3	L L L	L L H	L L L
21.....	1, 2, 3	L L L	L L L	L L L
23.....	1, 2, 3	L L L	L L L	L L L
24.....	1, 3	L L	L L	L L
25.....	1, 2, 3	L L L	H M L	H H L
26.....	2	M	H	H
27.....	1, 2	L L	L L	L L
28.....	1, 2, 3	M H H	M H M	H H H
29.....	1, 2, 3	H M M	H M H	L H L
30.....	2	H	H	H
31.....	2	L	L	L
32.....	1, 2	H L	L M	H H
33.....	2	L	M	H

Discussion of Table 7.

A glance at Table 7 reveals the fact that in several cases a correlation between soil and pasture values can hardly be said to exist. In some instances the explanation is at hand—different stages of growth of the pasture—as a glance at Table 1 will reveal. For instance, in area 3, third survey, the pasture grown on a soil, medium in phosphorus, showed a low figure for phosphorus, but consisted of old dry mature grass. In area 4 a high soil phosphorus produced grass just below the high margin, hence the designation medium.

The two anomalies in areas 17 and 20 are due to the fact, according to Table 1, that the pasture was green in both cases and, therefore, medium to high in phosphorus although growing on soil of low phosphorus content. On the whole, however, the correlation between soil and pasture values is remarkable. It must be remembered, that the periods under consideration—May, 1930, May and October, 1931—being those of poor feeding on account of winter and drought in a number of areas and as practically no new growth existed, favoured low values for phosphorus in pasture, thereby more easily establishing a correlation with low values for soil phosphorus. A comparison

between these two sets of values in summer will be interesting when mainly green grasses are present and stage of growth probably the important factor which will determine the phosphorus content of the pasture. A correlation under those conditions can hardly be expected to exist.

2. COMPARISON OF THE THREE METHODS OF STUDYING PHOSPHORUS DEFICIENCY.

The values for blood phosphorus agree remarkably well with those for pasture if the difficulty of obtaining for analysis representative samples of pastures actually eaten by stock be borne in mind. Then, too, there is the period of hydrolysis in the trichloroacetic acid solutions of the blood which considerably complicates the result. Still, a correlation of pasture with blood values with good technique is practically a certainty and these two methods of studying the problem of phosphorus deficiency in livestock, i.e. blood and pasture analyses respectively, have the advantage over soil analysis in that they deal directly with the animal or with its food, provided human attempts to select from the pasture samples of the grazing "eaten" by stock are successful. In laboratory experiments, where the intake of phosphorus is known and can be controlled, a direct relation is brought about within a few days and continues to exist between a low phosphorus intake and low inorganic phosphorus in the blood. The same relation holds on Armoedsvlakte pasture poor in phosphorus as a glance at the figures for inorganic phosphorus in the blood given by Malan, Green and Du Toit (1927) and Malan and Bekker (1930) will reveal. In spite of a low phosphorus content of the soil, figures for phosphorus in the pastures will be high at certain seasons of the year when abundant green growth is available and it is just at this period that a correlation of soil values with pasture values will most probably fail, as several results in Table 7 suggest. Blood values for phosphorus on the other hand will rise and fall with a greater and a decreased phosphorus intake respectively. It is for the obvious reason that blood analyses throw light upon the phosphorus equilibrium in the animal body and entails very little labour and, therefore, holds a not inconsiderable advantage over soil and pasture analyses that this phase of the work is rapidly extending and will again be reported on in the near future.

V. SUMMARY.

1. An account of the work done since March, 1930, on the study of mineral deficiencies in South African pastures is presented.

2. The problem is being studied from three aspects, viz., soil, pasture, and blood analyses, although the last method applies only to phosphorus at present.

3. Phosphorus, calcium, magnesium, sodium, potassium, chlorine, fibre, crude protein and carbohydrate plus fat are included in the analyses of the pasture.

4. Surveys, which entail the sending in of samples of soil, pasture and blood by about 40 Government field veterinary officers, are carried out at the four seasons of the year, while provision has been made for the analyses of blood samples at more frequent intervals.

5. The plan of the work includes a study as outlined above on samples obtained from the same area for each survey for the first few years in order to find the main effects of climatic conditions, such as a variation of rainfall on the composition of soil, pasture and blood, before any other areas are included.

6. Blood samples are drawn from mature cattle preferably, trichloroacetic acid added to precipitate the blood proteins, and to prevent decomposition, then forwarded to Onderstepoort and analysed for inorganic phosphorus on arrival.

7. Species of grass samples are sent in separately; these are identified and classified as green, mature, mixed, mixed mainly mature or green, mixed—practically all mature or green, and old respectively, before analysis.

8. The probable correlation of values obtained by the three methods employed—soil, pasture and blood analyses respectively—is discussed.

9. Stage of growth undoubtedly plays a very important part in determining the chemical composition of pasture. Hence plot experiments have been begun where a number of grass species have been planted in separate plots and are analysed at regular intervals. In addition, these plots provide material at any stage of growth decided upon for the determination of the differential distribution of minerals, etc., in plants.

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REFERENCES.

- HUTCHEON, D. (1895-1903). Report of the Colonial Veterinary Surgeon. Cape of Good Hope.
- JURITZ, C. F. (1910). A Study of the Agricultural Soils of the Cape Colony. Publ. *Cape Times, Ltd.*
- MALAN, A. I. (1930). The Increase of Inorganic Phosphorus due to Hydrolysis in Solutions of Blood. *16th Rept. Dir. of Vety. Serv.*, pp. 287-292.
- MALAN, A. I., AND BEKKER, J. G. (1931). Inorganic Phosphorus in the Blood of Pregnant Heifers. *17th Rept. Dir. of Vety. Serv. and Anim. Indust.*, pp. 433-438.
- MALAN, A. I.; GREEN, H. H.; AND DU TOIT, P. J. (1928). Composition of Bovine Blood on Phosphorus Deficient Pasture. *Jnl. Agric. Sc.*, Vol. 18, pp. 376-383.
- MALAN, A. I., AND VAN DER LINGEN, G. W. B. (1931). The Micro-determination of Some Inorganic Elements in Blood and Vegetation. *17th Rept. Dir. of Vety. Serv. and Anim. Indust.*, pp. 443-452.
- ORR, J. B. (1929). Minerals in Pastures. Publ. H. K. Lewis & Co., London.
- STAPLES AND TAYLOR (1926-30). Studies in Veld Management. *Sc. Bull.* Nos. 49 and 91, Union Dept. of Agric.
- STAPLETON *ET AL.* (1927/32). Welsh Plant Breeding Station, Aberystwyth Series.
- STEENKAMP, J. L. (1930). Micro-chemical Analysis of Soils. Proceedings and Papers of the Second International Congress of Soil Science. Moscow, 1930.
- VAN WYK, D. J. R. (1932). Feeding Value of Grasses. Public Lecture at Meeting of Chemical Institute, Pretoria.
- WOOD, T. B. (1911). A Course of Practical Work in Agricultural Chemistry for Senior Students. Cambridge University Press.
- WOODMAN, H. E., *ET AL.* (1926-1932). Series on the Nutritive Value of Pasture. *Jnl. Agric. Sc.*

Cystine and Sulphur Content of Bushes and Grasses in a Karroid Area (Fauresmith).

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THE present paper is the outcome of a query put to the author by the Director of Veterinary Services of the Union as to whether the leaves of plants contain cystine to any large extent, the question being prompted by Brailsford Robertson's 1929 Report of the Work of the Animal Husbandry Division of the Commonwealth of Australia, which mentions that cystine cannot be built up by the animal body but must be supplied by the plant food. At the time literature on the subject was very limited, and it was therefore decided to investigate the point, more particularly on the indigenous bushes of the Fauresmith area.

During the course of the work, Aitken's (1930) paper appeared, based also on Robertson's report, and after the experimental part had been completed, Evans's paper (1931) confirmed Aitken's results for England. Both these papers deal with grasses; the results of the two investigations will be discussed later on.

The Fauresmith laboratory is situated in the S.W. Free State at an altitude of 4,700 feet, on the slope of a kopje facing east. The soil of the Reserve is shallow, sandy, the sand being rather coarse, and poor in nitrogen. The pH value shows it to be alkaline, but it is not "brak", although a number of boreholes in the near neighbourhood yield brak water.

A full description of the meteorological and edaphic features of Fauresmith will be given in a later paper. Here only the following will be mentioned:—

The average yearly rainfall on the Reserve is 13.25 in. or 34.45 cm. There are two rainy periods, the earlier falling somewhat irregularly in the months September to December and the second from January to March.

The rain often falls in heavy gushes, causing a lot of erosion; on the other hand dry storms with little or no rain are frequent especially in the spring and early summer. The period from April to the end of September is very dry, and even in the "rainy" season long dry spells are frequently encountered. The evaporation is increased by strong winds especially from August to November. The temperature range is wide, very high temperatures occurring from September to March, and very low, with heavy frosts, in the winter. Even during the summer months it may happen that the temperature falls nearly to freezing point, following a day with a maximum of 35° (C).

MATERIAL USED.

The following plants were tested for cystine: *Salsola aphylla*, *Salsola glabrescens*, *Atriplex nummularia*, *Atriplex capensis*, *Tetragonia arbuseula*, *Mesembrianthemum hamatum*, *Digitaria eriantha* var. *stolonifera* and *Eragrostis, curcula* var. *conferta* for the season 1929/30 and *Euryops multifidus*, *Tripteris pachypteris*, *Pentzia incana forma* and *Chloris gayana* for the season 1930/31.

A few samples from other habitats were also tested—a stoloniferous *Digitaria* from Malmani Oog (W. Transvaal) and some Namaqualand plants characterised by a high sulphur content.

Plants are sampled regularly at the Reserve, at monthly intervals over thirteen months, so that (air dried) material was available for a systematic investigation of the seasonal variations, if any, in the cystine content.

METHOD FOR CYSTINE DETERMINATION.

Different methods were first tried to make quite sure that an eventual negative result was not due to an unsuitable extraction.

A first extraction was based on the method of Sasaki (cited from Abderhalden II. p. 575) by extracting finely ground plant leaves with alkaline alcohol and precipitating the protein by neutralisation with HCl. The sulphur content of the precipitated protein was determined gravimetrically. As it appeared that the amounts were very small, the method was abandoned and a method was worked out, based on the method of determining cystine in wool. It may be mentioned here that the two methods gave the same results. The method finally adopted was the following:—100 gm. of finely ground *air dried* plant material was digested in a 1,000 c.c. Pyrex flask with 500 c.c. 20 per cent. hydrochloric acid (1:1) for at least 8 hours on an electrical sandbath under a reflux cooler. At the beginning the digestion was extended to 20 hours, but it soon appeared that the same results were obtained with the shorter digestion. After that time the contents of the flask were filtered through a large Schleicher & Schüll paper No. 597. The liquid as well as the undissolved plant material were very dark coloured. After a thorough washing with hot water, the residue was rejected; in some cases a sulphur-determination was done on it. About 25-30 gm. of black residue was obtained. The solution was if necessary evaporated to a smaller volume, cooled, and under cooling in ice-water, solid caustic soda added until pH4 was reached. Bromphenolblue was used as indicator and did excellent service in spite of the solutions being so dark. Congo red which is recommended in several handbooks, was not found as satisfactory. Towards pH4 a brown precipitate came down. 20 c.c. Acetic acid was then added, care being taken that the pH did not rise above 4. This solution was left standing for several days, during hot weather in a Frigidaire, and the precipitate then filtered off, the liquid being discarded. Some determinations of sulphur were done on the liquid, and it was often tested with lead acetate in alkaline solutions to make sure that no cystine was left behind, although as Evans (1931) points out, this

test, which is recommended in biochemistry handbooks, is ambiguous, other organic sulphur compounds also precipitating lead sulphide under the circumstances. The same impression was obtained in the present work, when although small amounts of lead sulphide were obtained, no more cystine could be obtained from the solution by any chemical means.

The precipitate was dissolved in 5 per cent. HCl, boiled up, and about a teaspoonful of charcoal added, which was generally sufficient to decolorize the solution immediately, the charcoal being then filtered off. The solution was then treated with strong Ammonia (1:1 by vol.), a voluminous white precipitate coming down towards pH4. Bromphenolblue was again used as indicator. Acetic acid was again added, the pH being kept below 4.5. After some hours a crystalline looking precipitate settled and was filtered off. The solution was then if necessary evaporated to a small volume, alcohol added, and allowed to stand a few days in the *Frigidaire*; generally a second small precipitate crystallised out, which was filtered off. Tests on the filtrate with lead acetate in alkaline solution never gave positive results at this stage. All the reagents, filter paper, etc., used in this investigation were of course tested for the presence of cystine.

The two precipitates which were supposed to contain cystine were treated in different manners, till finally the Sullivan reaction—with some modifications described below—was adopted for the determination of cystine.

At the start about fifty precipitates were thoroughly searched under the microscope for cystine. A rather uniform crystalline precipitate was found, very similar in appearance in all the precipitations, in which, in nearly every case (except for the grasses) the typical hexagonal plates of cystine were found embedded. The proportion of cystine to the rest of the precipitate was perhaps only 1 to 200 in most cases, as shown by determinations made on several of the precipitates. In some exceptional cases where larger amounts of cystine were present it was possible to separate it mechanically, the cystine being much the heavier portion. The sulphur content of the cystine was 26.6 per cent., the "crystal sand" proved to contain none. In cases where it was not possible to separate the cystine, it was determined by an adaptation of a colorimetric method.

It was thought of interest to find out what the precipitate obtained under the above precipitating conditions really consisted of, apart from cystine, so a qualitative analysis was made according to Treadwell and Hall (1924). Silicates were the only anions found, but the following cations were present: Fe, Mg in small quantities and Na and Al in large quantities. Occasionally other amino acids could be detected under the microscope, to judge from their crystal form leucine, glycol and tyrosine (?), but as they were present in only very small amounts they were not more closely identified.

Before going on to the discussion of the details of colorimetric methods, another important point must be mentioned. As stated above, only very small quantities of cystine were found under the microscope. Though it was not considered likely to be the case, yet the possibility was feared of the cystine being destroyed during the process, broken down entirely during the extraction. To make quite sure on the point, 1 gm. of Merck's G.R. or Hoffman La Roche (Basle) cystine was added to the plant powder for digestion, and was invari-

ably regained at the end of the process, fractions of milligrams only being missing. It was no doubt an expensive way of testing the point, but was worth the assurance gained. As the cystine was recovered, it could be used over again. As a matter of fact the cystine crystals obtained by this process were better than any of the original material used. Very likely the cystine hexagonals break up into crystal sand when stored for some time; cystine crystals obtained by the author kept their form for at least a year.

It was thought of using Folin's method (1922) to determine the cystine, but it soon appeared that the values obtained were too high and varied according to the amount of total white precipitates (silicate & cystine) and not with the amount of cystine. Something contained in the rough precipitate developed a deep blue colour with Folin's reagent, thus the blank was much too high to determine small amounts of cystine with any accuracy. It seems that Evans had similar difficulties. At any rate, finally Sullivan's paper was obtained and his method adapted to meet the requirements of a plant analysis. The revised method of Folin and Marenzi came into the hands of the author too late to be tried out.

At first Sullivan's prescription was followed accurately, the rough precipitate being dissolved in 0.1N HCl, so that a lot of the silicate did not dissolve and could be filtered off. Then 2 c.c. of freshly prepared 1 per cent. sodium cyanide in 0.8N sodium hydroxide was added, care was taken to add 2 c.c. of 5N sodium hydroxide before the reducing agent was added—if this were omitted no colour whatever appeared. Then the 1 c.c. of 0.5 per cent. fresh aqueous solution of 1:2 naphthoquinone-4-sodium sulphonate was added, mixed and finally the 5 c.c. of 20 per cent. anhydrous sodium sulphite in 0.5N sodium hydroxide was added, and allowed to stand for 30 minutes and treated exactly as Sullivan prescribes further. After the final volume had been read, comparison was made with the standard of 1 to 3 mgr. cystine treated in exactly the same way, due allowance being made of course for the aliquot of the unknown. The colours could scarcely be matched and accurate readings were impossible. The colour of the unknown tended towards olivegreen-red. The puzzle was that although surely cystine was present in the solution of the unknown, how could the colours be matched?

The following method was tried out to overcome the difficulty which after all seemed to work very well. Known amounts of cystine dissolved in the hydrolysates of the plants gave exactly the same colour shade as the unknown simply treated with the Sullivan reagents, only of a different depth. Why not dissolve the standard in a known amount of plant hydrolysate of the unknown and compare it in the colorimeter with the same amount of the unknown alone? If the unknown contained any appreciable amount of cystine, the reading would be possible. A Bausch and Lomb colorimeter was used, and a formula was calculated for the reading.

If a is the standard in mgr, b the setting (mm.) of the standard, c the reading of the unknown (mm.), then x the amount of the unknown in mgr. can be calculated as follows:—

$$x = \frac{(a - x) b}{c} \quad \text{whence} \quad x = \frac{ab}{(c - b)} \quad (1)$$

In dissolving the unknown in 0.1N HCl, due allowance must be made in formula (1) for the quantities taken in the colorimetric determination. If e.g. there were 40 c.c. solution of the unknown, and 10 c.c. each were taken for standard and unknown, formula *a* had to be multiplied by 4 or generally speaking formula (2) is obtained, where *d* means the part of the aliquot to total amount of the unknown

$$x = \frac{a b d}{(c - b)} \quad (2)$$

The readings were checked with different standards, as the amount of the unknown allowed it, and very satisfactory results were obtained. This procedure was therefore finally adopted. In "negative" unknowns only a yellow colour was developed which could not be matched with the standard in the hydrolysates. Amounts under 1 mgr. in 100 gm. plant material could not be read accurately and are marked as traces in the tables. Generally 10 c.c. of the hydrolysate were taken for each of unknown and standard. For very weak solutions of the unknown, the whole unknown solution was just divided into 2 portions. For very concentrated unknown solutions 1 c.c. was sufficient to develop an intense colour with the reagents. The final volume of the standard and the unknown, after treatment with the reagents, was always equal, generally 30 c.c.

Owing to the lengthy analyses, only a few could be done in duplicate. These few agreed so well in the results, that the determinations were not all duplicated.

METHOD FOR SULPHUR DETERMINATION.

As it was thought possible, although not likely, that there was a relationship between cystine and the sulphur content of the plants, a total sulphur determination was done on all the plant samples. The method of Frear (1930) was found exceedingly useful for the purpose. As most Fauresmith plants contain large amounts of sulphur, 0.5-2.0 gm. of the ground plant powder were used according to the amount of sulphur expected. The barium sulphate was precipitated hot. The precipitate was filtered through Jena Glass Filter No. 4. All analyses were done in duplicate.

For the protein determination the usual Kjeldahl method was used.

RESULTS.

Table 1 shows the protein and sulphur content of all the monthly samples in the particular season as well as the cystine content of the selected samples. As can be seen at the first glance, none of the three compounds is constant through the season nor are the fluctuations for the different species uniform.

The Protein Content.

The protein content, being the best known, may be considered first. There is no doubt that the rainfall has a great influence on the protein content, on the other hand some plants seem to be much more independent of the rain than others.

TABLE 1.—SULPHUR, PROTEIN, AND CYSTINE CONTENT OF FAURESMITH PLANTS IN THE SEASON. 1929-30.

Date.	<i>Salsola Aphylla.</i>			<i>Salsola Glabrescens.</i>		
	Sulphur as Per- centage SO ₄ of Dry Matter.	Protein as Per- centage of Dry Matter.	Cystine as Per- centage of Dry Matter.	Sulphur as Per- centage SO ₄ of Dry Matter.	Protein as Per- centage of Dry Matter.	Cystine as Per- centage of Dry Matter.
1929.						
May.....	—	—	0·025	1·88	17·89	0·014
June.....	—	—	—	—	—	—
July.....	—	—	—	—	—	—
August.....	—	—	—	—	—	—
September.....	4·00	18·81	0·025	1·94	21·48	0·013
October.....	4·72	18·99	0·006	3·20	25·46	0·039
November.....	4·21	26·25	0·010	3·63	22·84	0·008
December.....	4·86	23·72	0·012	3·15	22·31	—
1930.						
January.....	4·28	21·52	0·001	1·63	22·31	0·002
February.....	3·63	20·74	0·065	2·24	19·42	0·002
March.....	3·64	19·95	0·034	2·23	19·42	—
April.....	3·31	17·31	—	3·30	13·83	—
May.....	3·52	18·55	0·004	2·00	15·75	0·018
June.....	3·21	19·95	—	2·43	15·23	0·013
July.....	3·30	19·56	0·036	2·89	14·26	—
August.....	3·43	—	—	2·93	13·26	0·009
September.....	2·70	18·33	0·035	2·45	12·43	0·017
October.....	3·26	16·31	0·044	3·00	12·86	—
November.....	—	—	—	2·49	20·39	0·021

TABLE 1.—SULPHUR, PROTEIN, AND CYSTINE CONTENT OF FAURESMITH PLANTS IN THE SEASON 1929-30—continued.

Date	<i>Atriplex Capensis.</i>			<i>Atriplex Nummularia.</i>		
	Sulphur as Per- centage SO ₄ of Dry Matter.	Protein as Per- centage of Dry Matter.	Cystine as Per- centage of Dry Matter.	Sulphur as Per- centage SO ₄ of Dry Matter.	Protein as Per- centage of Dry Matter.	Cystine as Per- centage of Dry Matter.
1929.						
May.....	2·76	21·92	—	—	—	—
June.....	—	—	—	—	—	—
July.....	—	—	—	—	—	—
August.....	—	—	—	—	—	—
September.....	1·93	26·77	—	2·86	25·59	0·004
October.....	1·96	24·19	0·025	2·64	22·31	—
November.....	2·42	23·80	0·037	2·26	22·40	0·006
December.....	1·44	24·06	0·017	2·14	23·19	0·027
1930.						
January.....	2·23	14·87	0·031	2·50	15·75	0·006
February.....	2·35	21·17	—	2·59	21·00	0·009
March.....	1·24	23·19	0·007	2·44	23·19	—
April.....	1·24	21·53	—	2·79	20·83	0·021
May.....	1·64	22·88	0·036	2·95	20·69	0·036
June.....	1·61	22·58	0·010	2·51	20·21	0·018
July.....	1·60	18·38	0·034	2·98	18·90	0·016
August.....	1·87	22·11	—	2·44	18·55	trace
September.....	1·70	—	0·039	3·08	19·16	—
October.....	1·68	26·47	trace	2·94	17·81	0·011

TABLE 1.—SULPHUR, PROTEIN, AND CYSTINE CONTENT OF FAURESMITH PLANTS IN THE SEASON 1929-30—*continued*.

Date.	<i>Tetragonia Arbuscula.</i>			<i>Mesem. Hamatum.</i>		
	Sulphur as Per- centage SO ₄ of Dry Matter.	Protein as Per- centage of Dry Matter.	Cystine as Per- centage of Dry Matter.	Sulphur as Per- centage SO ₄ of Dry Matter.	Protein as Per- centage of Dry Matter.	Cystine as Per- centage of Dry Matter.
1929.						
May.....	—	—	—	—	—	—
June.....	1·52	18·46	0·005	—	—	—
July.....	—	—	—	—	—	—
August.....	—	—	—	—	—	—
September.....	1·34	21·44	0·039	0·60	10·6	—
October.....	1·17	19·69	0·002	0·76	9·98	—
November.....	1·30	22·92	0·014	0·86	11·46	0·013
December.....	1·50	23·36	—	0·37	9·62	—
1930.						
January.....	1·10	21·52	0·016	0·40	10·24	0·023
February.....	1·07	20·30	0·050	0·32	10·32	0·012
March.....	1·23	21·09	0·097	0·44	11·99	0·010
April.....	1·20	20·75	—	0·42	10·50	—
May.....	1·20	18·99	0·015	0·53	9·01	0·016
June.....	0·88	16·19	0·008	0·42	9·76	—
July.....	0·78	12·60	0·018	0·42	8·75	0·003
August.....	0·86	12·16	0·1852	0·41	9·45	—
September.....	1·18	18·86	—	0·39	8·36	0·018
October.....	1·27	22·75	0·0152	0·43	11·20	0·009

TABLE 1.—SULPHUR, PROTEIN, AND CYSTINE CONTENT OF FAURESMITH PLANTS IN THE SEASON 1929-30—*continued*

Date.	<i>Digitaria Eriantha Stolonifera.</i>			<i>Eragrostis Curvula Var. Conferta.</i>		
	Sulphur as Per- centage SO ₄ of Dry Matter.	Protein as Per- centage of Dry Matter.	Cystine as Per- centage of Dry Matter.	Sulphur as Per- centage SO ₄ of Dry Matter.	Protein as Per- centage of Dry Matter.	Cystine as Per- centage of Dry Matter.
1929.						
May.....	—	—	—	—	13·12	0·0
June.....	—	—	—	—	—	—
July.....	0·39	7·96	—	—	—	—
August.....	—	—	—	—	—	—
September.....	0·81	20·48	0·033	—	—	0·0
October.....	—	14·96	—	0·36	10·59	—
November.....	0·69	13·91	0·010	0·67	12·51	0·0
December.....	0·51	13·65	—	0·62	15·75	0·0
1930.						
January.....	0·55	12·60	—	0·50	13·30	0·0
February.....	0·33	10·15	0·009	0·37	12·69	—
March.....	0·51	10·06	—	0·38	10·50	—
April.....	0·55	8·09	—	0·40	11·02	—
May.....	0·46	8·21	0·009	0·35	8·49	0·0
June.....	0·06	5·43	—	0·19	5·25	—
July.....	0·38	5·67	0·006	0·38	4·73	0·0
August.....	0·38	5·25	—	0·45	4·81	—
September.....	0·39	16·36	—	0·60	12·95	0·017
October.....	0·78	13·38	0·002	0·58	14·61	0·0

TABLE 2.—RAINFALL AT FAURESMITH DURING THE TIME OF THE INVESTIGATION IN INCHES.

	1929.	1930.	1931
January.....	2.21	2.87	2.82
February.....	0.0	1.20	1.18
March.....	2.74	1.69	2.53
April.....	0.48	2.34	2.38
May.....	0.46	0.17	0.0
June.....	0.62	0.38	0.05
July.....	0.63	0.0	0.88
August.....	1.17	0.99	0.05
September.....	3.51	0.0	0.0
October.....	0.42	0.89	1.44
November.....	0.29	0.52	2.955
December.....	2.62	2.00	—

From the rainfall table it is obvious that the season July, 1929, to June, 1930, was a good one, there being one spell of drought after heavy rains from the middle of October to the beginning of December. The season July, 1930, to June, 1931, was much less fortunate, as there was insufficient rainfall from September to the end of December, the late season, however, was favoured with rain.

From a comparison of the figures for protein with the rainfall it is evident that no correlation exists for the succulent *Mesem. hamatum*. The two grasses show the usual seasonal variation, high values in spring decreasing continuously towards the late summer where a rapid fall takes place and nearly constant values through the winter. *Eragrostis* has its highest values after good rains in the early season, a late season's rain raises its protein content but not very much. In winter for neither *Digitaria* nor *Eragrostis* leaching takes place owing to the lack of rain and heavy dew. *Digitaria* has its highest protein content in September, 1929, when the highest monthly rainfall occurred. No effect of the second rainy period is visible unless it is just veiled by a less rapid seasonal decrease. The two *Atriplex* and *Tetragonia* always have their highest protein content after heavy rains. The heavy rains in January, 1930, fell after the January samples of the plants had been collected, hence the low values, at the time of heaviest evaporation. Neither *Salsola aphylla* nor *Salsola glabrescens* seem to depend very much on the rain, *aphylla* having its highest value in the drought period, and *glabrescens* in 1930 after heavy grazing in the dry November. For both plants but especially for *aphylla*, it is characteristic that even in winter their protein value is not low. All the four *Chenopodiaceae* have their lowest value in spring or summer, *Tetragonia* and the *Mesembrianthemum* in winter like the grasses. It means that the winter does not inhibit growth in the *Chenopodiaceae*.

The Sulphur Content.

It is quite obvious that the investigated plants differ very much in their sulphur content. The two grasses and the *Mesembrianthemum* are by far the lowest. Then follows *Tetragonia* which like the grasses has its lowest value in winter. *Atriplex capensis* which comes next has its lowest sulphur content in March and April. *Atriplex nummularia* and *Salsola glabrescens* have both a much

higher sulphur content and show their minimum in sulphur during the rainy season, and their maximum in spring, independent of prevailing rain or not. The second maximum in the rainy April, 1930 for *Salsola glabrescens* and for *Atriplex* is characteristic. *Salsola aphylla* has the highest sulphur content, the highest values during and after the heavy rains in 1929 and the lowest value in the rainless September, 1930. There is no doubt that *Salsola aphylla* contains a good deal of inorganic sulphur, whilst in the grasses most of the sulphur is present in organic form. *Salsolas* on brak soil can store a very high percentage of sulphur, either as calcium (and magnesium) salt or as sodium salt.

Some of the samples for the season 1930/31 were obtained in a different way, in the case of *Euryops* and Rhodes grass, different strips being cut at monthly, bi-monthly and three-monthly intervals for analysis. This meant that bulk was sacrificed to quality and it was not possible to make as many cystine analyses as with the other plants. As can be seen from Table 3 the protein content of *Euryops*, a winter flowering plant, is highest in May but after this the plant could not stand the monthly cutting, and only one more figure could be obtained. Rhodes grass had also its highest protein content in the early winter, later on owing to the heavy frosts it died entirely down. *Tripteris pachypteris* is at its best in summer, but grows very well in the early winter and flowered a second time in June. Neither *Pentzia incana* nor *Tripteris* have outstanding values in protein, but on the other hand *Tripteris* never decreases its protein content very much, although *Pentzia* does so during drought. *Pentzia* has its best values after the rainy period in January and March.

With regard to the sulphur content, *Tripteris* is the leader on Fauresmith soil, which is not brak. Values to nearly 6 per cent. are reached in spring, the lowest value falling during and after the heavy January rains. *Pentzia* does not contain much sulphur and does not vary its sulphur content to any extent. *Euryops* shows considerably more and varies in rather an irregular way, this may be due to the close cutting. Rhodes grass has for a grass a fairly high sulphur content, of course not high compared to the bushes; its maximum is in winter, the lowest values being found in the rainy season though the differences on the whole are not large.

From the foregoing it is evident that protein and sulphur content do not vary uniformly for all the species. Some of them have their lowest sulphur content in the rainy season (*Tripteris*, Rhodes grass, *Atriplex nummularia*, *Atriplex capensis* and *Salsola glabrescens*). The others have it in the winter (*Salsola aphylla*, *Tetragonia*, *Mesembrianthemum hamatum*, *Digitaria eriantha stolonifera* and *Eragrostis conferta*). But most of these plants show a second relative minimum in the other period as well.

With regard to the protein content, three types of plants may be distinguished, (a) a group with a high protein content in early summer decreasing towards autumn: *Salsola aphylla* and *glabrescens*, *Mesem. hamatum*, *Eragrostis conferta* and *Digitaria eriantha stolonifera*. (b) The second group which is spread over all the dry parts of South Africa has two distinct maxima of protein content, one early

TABLE 3.

SULPHUR, PROTEIN, AND CYSTINE CONTENT OF FAURESMITH PLANTS IN THE SEASON 1930-31.
ALL VALUES AS PERCENTAGE OF DRY MATTER.

Date.	<i>Euryops Multijugus.</i>			<i>Tripteris Pachypteris.</i>			<i>Peutzia Incana Form.</i>			Rhodes Grass.		
	Sulphur.	Protein.	Cystine.	Sulphur.	Protein.	Cystine.	Sulphur.	Protein.	Cystine.	Sulphur.	Protein.	Cystine.
1930.												
November.....	1.87	9.89	0.0142	5.86	—	—	0.90	9.98	0.0107	1.13	9.01	0.0
December.....	3.14†	14.70†	—	5.56	10.06	0.0126	0.85	6.56	0.0121	1.00†	8.44‡	—
1931.												
January.....	1.86†	21.75‡	—	3.36	15.66	0.0237	0.85	10.76	—	0.83†	5.95‡	—
February.....	1.76†	21.32‡	—	4.12	15.53	0.8065	0.91	13.91	—	0.89†	6.82‡	0.002
March.....	1.26†	21.32‡	—	4.12	15.53	0.0107	0.88	10.68	0.005	0.78†	8.40‡	0.0
April.....	1.64†	28.32‡	—	4.32	15.31	0.0017	1.06	13.21	—	1.13†	9.67‡	—
May.....	2.32†	29.66†	{ 0.0049† 0.0029 0.0009*	4.06	15.75	—	0.87	13.30	0.020	1.40†	9.10†	0.0349
June.....	No material available.			4.06	13.47	0.0894	0.77	10.76	0.020	1.29†	10.02†	0.0349
July.....	No material available.			5.02	11.44	0.0052	0.80	9.89	0.018	1.10	6.41	0.008
August.....	2.13†	No material.	0.0012	5.08	11.46	—	0.74	9.32	0.007	—	No material.	—
September.....	No material.			5.08	11.33	0.018	0.85	7.93	0.006	—	No material.	—
October.....	No material.			5.62	11.99	—	1.07	7.69	—	—	—	—

* Strip cut once a year. S. content. 1.02 % Protein, 14.53 %

† Strip cut twice a year. S. content. 2.38 % Protein, 13.04 %

‡ Cut every month.

§ Cut every second month.

in the season and another after the good rains in January. The two *Atriplex*, *Tripteris*, Rhodes grass and *Tetragonia* fall in this group. (c) The last group is the typical winter flowering plant *Euryops multifidus* with a maximum in winter. The maximal value in protein reveal at the same time a maximum in growth of the plant.

COMPARISON OF THE DECREASE OF SULPHUR AND PROTEIN CONTENT.

From Table 1 it can be seen that for *Digitaria* the sulphur content decreases in winter to less than 10 per cent. of its high spring value and to about 28 per cent. for *Eragrostis*, whilst the corresponding protein values are 24 per cent. and 31 per cent. respectively of the spring values. For the bushes the relation is quite different, as Table 4 indicates.

TABLE 4.

	Lowest Sulphur Content Expressed as Percentage of Highest.	Differ.	Lowest Protein Content Expressed as Percentage of Highest.
<i>Salsola aphylla</i>	62	+ 6	56
<i>Salsola glabrescens</i>	49	+ 4	45
<i>Atriplex capensis</i>	51	- 5	56
<i>Atriplex nummularia</i>	69	+ 7	62
<i>Tetragonia arbuscula</i>	51	- 1	52
<i>Euryops multifidus</i>	38*	+ 5	33*
<i>Tripteris pachypteris</i>	35	-29	64
<i>Pentzia incana</i>	70	+23	47
<i>Mesem. hamatum</i>	27	-43	70
Rhodes grass.....	56*	- 3	59*

* No winter values available.

With few exceptions the percentage decreases for sulphur and protein are much closer than for grasses, and therefore also much nearer than for Evans (1931 p. 811) samples. One exception is a *Mesembrianthemum*, of which the metabolism is still a closed book, another is *Pentzia incana forma* which varies but little in its sulphur content but a good bit in its protein content. At first sight it might be thought that on the whole there is a close relationship in the fluctuations of the sulphur and protein contents, as the percentage variations are so small. But it has to be remembered that only in a few cases are the lowest protein values found at the same time as the lowest sulphur values, neither are the maxima found at the same time (see especially *Euryops* and *Tripteris*) so that this table loses its convincing aspect and as in Evans' investigation it has to be concluded that there is no direct relation between sulphur and protein content. Table 5 expresses the sulphur values at the time of the lowest protein value as a percentage of the sulphur value at the time of the highest protein content and proves the aforesaid statement.

It can be seen that for a few plants the sulphur even increases as the protein decreases and in all other cases the protein content falls off to a much larger extent than the sulphur content. The differences are on the whole much larger than Evans' (1931) differences, only *Digitaria* and Rhodes grass come near his figures; this fact, however, seems entirely due to the different plants and surroundings.

TABLE 5.

	Sulphur Value at the Time of Lowest Protein Value Expressed as Percentage of S-value at the Time of Highest Protein Value.	Differ.	Lowest Protein Content Expressed in Percentage of the Highest.
<i>Salsola aphylla</i>	77	21	56
<i>Salsola glabrescens</i>	77	32	45
<i>Atriplex capensis</i>	109	53	56
<i>Atriplex nummularia</i>	87	25	62
<i>Tetragonia arbuscula</i>	57	5	52
<i>Euryops multifidus</i>	81*	48	33*
<i>Tripteris pachypteris</i>	154	90	64
<i>Pentzia incana</i>	93	46	47
<i>Mesem. hamatum</i>	89	19	70
<i>Eragrostis conferla</i>	73	49	24
<i>Digitaria eriantha</i>	47	16	31
Rhodes grass.....	78*	19	59*

* No winter values available.

THE CYSTINE CONTENT.

The cystine content may first be considered quite independently of the protein and sulphur contents, as to its order of size. A first glance at Tables 1 and 3 reveals that on the whole the cystine content is very small. A few exceptions will be discussed later.

If a calculation is made how much of such plants a sheep must eat to obtain its cystine content in 10 lb. of wool, the following figures are found:—

Weight of wool 10 lb. (German lb.)

Cystine in wool 13.0 per cent. = 1.3 lb. cystine = 650 gm.

A figure which is often met with in Tables 1 and 3 is 0.02 per cent. Cystine.

Taking this as basis, it would take 3,250,000 grams or 6,500 lb. (of 500 gm.) of dry plant food, to supply the 650 gm. of cystine in 10 lb. of wool, i.e. to produce a year's growth. As plants in the veld in South Africa contain about 50 per cent. water, 13,000 lb. of fresh matter per year would be required. The daily ration would amount to 35.6 lb., a figure which is about four times higher than the known daily intake of a sheep. With plants poorer in cystine (e.g. *Eragrostis*) the discrepancy would still be greater. From this deduction alone it seems unlikely that the sheep cover their cystine content from the cystine contained in the Karroo plants. It is quite likely that the cystine present in certain plants has a stimulating effect on the growth of wool and hair, as shown in many papers on the physiological effect of cystine (See Abderhalden 1930, p. 580 ff.), but to the author's mind they do not prove that cystine must necessarily be present in the food of the herbivorous animal. The mistake which is generally made is the assumption that cystine is the

only organic sulphur compound in the plant. Evans (1931) has pointed out that there must be other organic sulphur compounds present in the plant, from which cystine can be easily prepared by the animal body. In the present investigation, sulphur determinations were made on several of the hydrolysates or on the original hydrolysed material after the cystine had been removed. The original plant material, after being hydrolysed with a 20 per cent. hydrochloric acid for 8 hours contained only a small portion of sulphur with the exception of the grasses which contained more than half their sulphur content in this form. All other sulphur is found in the rejected hydrolysates or their wash water, which are still slightly acid after the cystine has been precipitated. The sulphur is mostly in S-II form. The plants do not contain much water soluble inorganic sulphur, which in this separation would be found in the hydrolysate. This may be different for plants on brak soil.

Some determinations of "water soluble" sulphur were done. 1 gm. plant material was extracted with about 20 c.c. hot water on the water bath. To the surprise of the author the following high figures were obtained.

Date.	Plant.	Total Sulphur.	So-called Water Soluble Sulphur.
7. 2.31	<i>Tripteris pachypteris</i>	4.12	2.87
20. 10. 30	<i>Salsola aphylla</i>	4.72	3.00
20. 10. 30	<i>Tetragonia arbuscula</i>	1.18	1.18
20. 10. 30	<i>Digitaria eriantha</i>	0.78	0.77
20. 1. 30	<i>Digitaria eriantha</i>	0.55	0.39
20. 1. 30	<i>Tetragonia arbuscula</i>	1.10	1.12

The explanation of this phenomenon was that the water extraction reacted strongly acid against litmus and was by no means a "water" extraction. No further determinations were made after the solution was neutralised.

Table 6 shows a few figures obtained indicating the distribution of the different sulphur compounds in the Karroo plants. The inorganic insoluble sulphur is in a relatively smaller quantity than in Evans' investigation. Only the grass can readily be compared with his figures, for all the other plants the organic part of the sulphur seems to be considerably higher than the insoluble inorganic part.

TABLE 6.—DISTRIBUTION OF SULPHUR.

Plant.	Total SO ₄	Inorganic SO ₄ in Rejected Original Plant Material after Hydrolysis.	Inorganic and Organic SO ₄ in Rejected Hydrolysate and Washwaters after Precipitation of Cystine.	Cystine.
	°/°	°/°	°/°	°/°
<i>Atriplex capensis</i>	1.94	0.48	1.46	0.025
<i>Salsola glabrescens</i>	1.88	0.11	1.76	0.014
<i>Tetragonia</i>	1.52	0.11	1.41	0.005
<i>Digitaria</i>	0.39	0.22	0.17	0.009
<i>Atriplex capensis</i>	1.50	0.25	1.24	0.027
<i>Salsola glabrescens</i>	3.2	0.63	2.37	0.039

Table 6 shows that there is no doubt that large amounts of organic sulphur are present in the Karroo plants, but they are not present in the form of cystine.

If the order of size of the cystine present in the investigated plants is considered, it is obvious that the grasses contain the least. Once only could cystine be isolated at all from *Eragrostis conferta*. The amounts in *Digitaria eriantha stolonifera* are with the exception of the spring value slightly more than traces. Rhodes grass contains appreciable amounts of cystine only at the time of vigorous growth; at other times only traces or nothing at all could be found.

For the bushes it may first be stated that the cystine content is not constant throughout the year, but shows a lot of variations. One thing may be emphasized which does not show up in the table. Whenever the highest values were found, the plants were in a flourishing condition and showed vigorous growth. (See especially *Tripteris*). The exceptionally large amounts of cystine were only found for a very short time in each plant. If a plant showed two distinct periods of growth, a larger than usual amount of cystine was found in both cases (*Tripteris*, *Salsola aphylla*, *Atriplex capensis*, *Tetragonia*).

The cystine content even in the same genus varies a good bit. *Salsola aphylla* decidedly shows higher values than *Salsola glabrescens*, and *Atriplex capensis* generally contains more than *Atriplex nummularia*.

Some species are always decidedly poorer in cystine than others, neither *Euryops* nor *Pentzia incana* contain at any time more than small amounts, whilst *Tripteris pachypteris* holds the record of 0.8065 per cent. of cystine when it was at its best and 0.0894 per cent. when it was in its second growth period. *Tetragonia* is the only other plant which occasionally shows higher values. It reached 0.1852 per cent. in August, 1930, and 0.097 per cent. in March, 1930, both high values again when it was in prime condition.

When growth is at a standstill only traces of cystine are found in different plants (*Salsolas* in mid-summer, *Atriplex nummularia* before the rainy season, *Tetragonia* in the dry October, 1929, *Mesem. hamatum* and *Tripteris* in winter). The one exception to this rule seems to be *Euryops multifidus*, from which no larger amounts of cystine could be isolated in winter than at other times, perhaps the plants were too weakened by the continual cutting, as the bushes did not revive even after the heavy November rains in 1931. At any rate their growth was bad at their natural season of growth.

The question now arises whether there is a relationship between protein and cystine content. In some few cases the respective maxima fall at the same time, but in most cases they differ widely in time, so that it is not considered that a direct relation exists. If the two maxima fall together, it is probably rather accidental, as both maxima seem to be signs of vigorous growth, protein however of the time of development and cystine of the time of elongation—the more visible growth in length. These two periods may be separated in a dry climate by a considerable lapse of time or under favourable conditions the phases may follow one another very rapidly.

The second question as to a relationship between the total sulphur content and the cystine content has to be answered in the negative for the bushes. For Rhodes grass and *Digitaria* it seems that such a relation exists.

To see whether any relation between sulphur and cystine content exists under different climatic conditions, a few analyses were made of material from Namaqualand and grass from Bechuanaland. The results are given in Table 7. No more cystine was found and no relation between total sulphur and cystine exists.

TABLE 7.

	SO ₄ as Percentage of Dry Matter.	Cystine Content as Percentage of Dry Matter.
Leaves of <i>Ficus Burkei</i> . Pretoria.....	0.74	0.023
<i>Didelta carnosum</i> . Steinkopf. Flowers included. Sept., 1930.....	3.23	0.002
<i>Digitaria eriantha stolonifera</i> . Malmani oog. Febr., 1930.....	0.74	0.009

ROLE OF CYSTINE IN THE PLANT.

Up to now the cystine content of the plants has been considered more from the animal point of view, its existence in sufficient quantity or non-existence being discussed as to its probable relation to the wool. For the animal body the importance of cystine is evident. But what about its function in the plant? We actually know nothing about its physiological role in the plant. Whilst the influence of cystine on animals is two-fold, being necessary for wool and growth, and has been treated in many papers (see literature in Abderhalden, *Biochemisches Handlexikon*, 1930, p. 580 ff.), no literature exists on its function in the life of the higher plant. Macht (1929) alone investigated the influence of cystine on growth of seeds of *Lupinus albus* and found a small stimulation with d-Cystine, but none with l-Cystine in concentration 1:25000. To the author's knowledge no further literature is available.* Although the data are very scanty, the author ventures the hypothesis that cystine has a function in the growth of the higher plant; it is surely not accidental that the highest concentration of this amino acid was found in plants which were in flourishing condition and that grasses which have rather a struggle in the Fauresmith area and are never in really flourishing condition are so poor in cystine.

THE CYSTINE CONTENT OF KARROO PLANTS IN COMPARISON WITH THE CYSTINE CONTENT OF FODDER GRASSES.

Compared with Aitken's figures the total sulphur content of the investigated South African grasses is about the same and the negative results for the cystine content of two grasses agrees with his results.

*Whilst this paper is in print, the investigation of Grassmann, Schoenebeck and Eibeler appeared. According to them, Glutathione and cysteine, reduced derivatives of cystine, play a rôle as activators of proteases, and they think that Glutathione is in small quantities widely spread in animals and plants.

One difference certainly exists, that the best grass (under karroid conditions!), Rhodes grass, had decidedly a higher sulphur content than *Digitaria* or *Eragrostis*. Compared with Evans' figures, the present figures for total sulphur for grasses are of the same order of size; for the investigated bushes they are, of course, higher.

With regard to the cystine content of the grasses, Evans mentions slightly higher figures than has Rhodes grass (p. 820), but the figures are of the same order of size. It has already been mentioned that the two other grasses were never in prime growth condition—*Digitaria's* cystine content is at the best of times small, and for *Eragrostis* it is mostly nil.

The investigated bushes in a few cases show values considerably higher than the values for grass of Evans. But the average figures are of the same order of size, if anything rather lower than the few values published by Evans. Very low values are found when growth has stopped. Because of the varying optimum conditions for, and seasons of, best growth of the different plants, their lowest cystine values may occur at any time or season, or during drought. On the whole they may all be said to be very low in cystine for the greater part of the year, especially the grasses. From the point of view of the direct supply of cystine to the grazing animal, it is not likely that the isolated instances when more cystine than usual was found will compensate for the general deficiency, although they may have some influence on the growth of feeding sheep.

SUMMARY.

Determinations of total sulphur, crude protein and cystine were made on a number of different bushes and grasses growing in the karroid area of Fauresmith (S.W. Free State).

There is no relation whatsoever between the three compounds. The total sulphur of the bushes is considerably higher than that of grasses, although the plants did not grow on brak soil. Seasonal variations are discussed.

The cystine content of the investigated plants is low with very few exceptions. The highest cystine values were found in plants which showed vigorous growth. The grasses of the area are especially deficient in cystine.

LITERATURE REFERENCES.

-
- ABDERHALDEN, E. (1930). *Biochemisches Handlexikon*, Vol. 12, 1930.
- ABDERHALDEN, E. (1923). *Handbuch der biologischen Arbeitsmethoden*, Abt. I. *Chemische Methoden*, Part 7, 1923.
- AITKEN, HARRY A. A. (1930). Sulphur in Organic and Inorganic Combination in Pasture Grasses. *The Biochemical Journal*, Vol. 24, No. 2.
- EVANS, R. E. (1931). Studies on the Sulphur of Pasture Grass. I. The Cystine Content of Pasture Grass. *Journal of Agricultural Science*, Vol. 21, pp. 806-821.
- FOLIN, O., and LOONEY, J. M. (1922). Colorimetric Methods for the Separate Determination of Tyrosine, Tryptophane and Cystine in Protein. *Journal of Biological Chemistry*, Vol. 51, p. 421.

- FOLIN, O., and MARENZI (1929). An Improved Colorimetric Method for the Determination of Cystine in Proteins. *Journal of Biological Chemistry*, Vol. 83, pp. 103-108.
- FREAR, DONALD E. (1930). The Adaptation of the Benedict-Denis Method to the Determination of Sulphur in Plants. *Journal of Biological Chemistry*, Vol. 86, pp. 285-289.
- GRASSMANN, W.; SCHOENEBECK, O. v.; und EIBELER, H. (1931). Über die Aktivierung tierischer und pflanzlicher Proteasen durch Glutathion. Hoppe Seyler's Zeitschrift für physiologische Chemie, Vol. 194, pp. 124-136.
- HAWK, PHILIP B., and BERGEIM, OLAF (1927). *Practical Physiological Chemistry*. 9th Edit. London. Churchill.
- LOUW, J. G. (1931). The Preparation of Cystine. *17th Report of the Director of Veterinary Services and Animal Industry*, pp. 473-474. Union of South Africa.
- MACHT, DAVID I. (1929). Pharmaceutical Experiments on Leucins and Cystines. *Journal Pharmacol. exp. Therapeutics*, Vol. 36, pp. 243-250. Cited after *Chemisches Zentralblatt* 1929, Vol. II, p. 3033.
- MARSTON, HEDLEY R. (1928). The Chemical Composition of Wool, with Special Reference to the Protein of Wool-fibre. *Commonwealth of Australia. Council for Scientific and Industrial Research. Bull.* 38.
- MARSTON, HEDLEY R., and BRAILSFORD ROBERTSON, T. (1928). The Utilization of Sulphur by Animals. *Commonwealth of Australia. Council for Scientific and Industrial Research. Bull.* 39.
- RIMINGTON, CLAUDE (1930). The Colorimetric Determination of Cystine by means of the Uric Acid Reagent. *The Biochemical Journal*, Vol. 24 pp. 1114-1118.
- ROBERTSON, T. BRAILSFORD (1929). The work of the Division of Animal Nutrition for the year 1928-29. *Commonwealth of Australia. Council for Scientific and Industrial Research. Pamphlet* 16. Melbourne.
- SASAKI, R. (1928). Über einige Stickstoffbestandteile in den Blättern von Kuzu, der japanischen Arrow-root-Pflanze, *Pueraria hirsuta*, Matsum. *Bulletin Agricultural Society (Chemical)*, Japan 4. 1-5. Cited after *Chemisches Zentralblatt* 1929. Vol. II, p. 583.
- SULLIVAN, M. X. (1926). A Distinctive Test for Cystine. *U.S. Public Health Reports*, Vol. 41, pp. 1030-1056.
- SULLIVAN, M. X. (1929). Studies on the Biochemistry of Sulphur. II. Further Studies on the Distinctive Reaction for Cysteine and Cystine. *Public Health Reports*, Vol. 44, pp. 1421-1428.
- TREADWELL, F. P., and HALL, WILLIAM T. (1924). *Analytical Chemistry. Quantitative Analysis*. New York. Wiley & Sons.

The Effects of Sulphur on Merino Sheep and their Resistance to Potassium Cyanide Poisoning.

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CONTENTS.

- I. Introduction.
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I. INTRODUCTION.

SUBSEQUENT to the publishing of an article on "Geilsiekte" by the author ("Geilsiekte and its Detection in the Field", Jnl. S.A.V.M.A. Vol. 2, pp. 23 to 26), H. H. Curson, of Onderstepoort, handed me an extract from a report dated 6.4.21 to the Director of Veterinary Education and Research, Onderstepoort, in which he discussed the results of his investigations at Grahamstown into the cause of "Geilsiekte".

This report was never published and was unknown to me. I am, however, only too pleased to give prominence to it here, and append the following abstract:—

GEILSIEKTE (DUE TO HCN).

"*Dimorphotheca cuneata*, or 'Bietou'. See Expt. G. 33. Field observations without doubt show that this plant is the actual cause of one of the forms of so-called 'geilsiekte' of sheep. On testing the plant I find a cyanogenetic glucoside is present, and the symptoms seen by me correspond exactly with those produced in prussic acid poisoning. When at Bloemfontein a fortnight ago, I saw *Dimorphotheca* spp. growing outside the Show Ground and on

testing some material here by Guignard's method, again obtained a typical reaction. The Cape 'geilsiekte' that is most frequently seen is, to my mind, unassociated with parasitic infection. The conditions under which the malady is noticed are concisely thus: The veld is rapidly improving after the rains when a hot scorching day wilts the vegetation. The sheep which have been grazing since early morning begin about midday to show signs of distress. In many cases it will be found that they have just passed over or near *D. cuneata* (i.e. referring to Albany), when there is sudden mortality. Those sheep ill present the following symptoms: Appetite suddenly disappears, there is excitement (due to the preliminary stimulating action of the poison), respiration is faster but deeper, heart-beat is slower due to stimulation of inhibitory portion of vagus and blood pressure is raised. If only a small quantity of the plant has been consumed the animal probably recovers and the owner believes his crude remedies are actually responsible. If, however, a large amount of the herb has been eaten, the primary stimulation passes into paralysis and the vital centres of the medulla are involved. There is staggering, irregular respiration and cardiac action, the animal falls and convulsions follow; coma supervenes and death as a rule takes place within 6 hours from onset of symptoms. The post-mortem appearances are those of respiratory paralysis, and tympanites soon follows from fermentation of green food in stomachs. 'Geilsiekte' may follow after grazing on wilted 'kweek' (*Cynodon dactylon*), or probably other plants, and I feel confident that one form at any rate is due to prussic acid poisoning resulting from the ingestion of *D. cuneata* (2)."

In the 17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, the results of an experiment, in which different groups of sheep were dosed weekly with 1.25 drachms (5.0 grams), 3.75 drachms (15 grams) and 7.5 drachms (30 grams) of sulphur respectively, were published. This experiment was continued for another year and it is proposed to discuss the results in the following paragraph.

II. RESULTS OF EXPERIMENTS CONDUCTED AT ONDERSTEEPOORT.

(A) THE EFFECTS OF SULPHUR ON THE SHEEP.

[*Corrections.*—In the article appearing in the 17th Report of the Director of Veterinary Services, pp. 481-492, Sheep 21643 (Table 3) has been wrongly recorded as 21642 and the age of Sheep 22986, 24733 and 24418 (Table 4, Controls) recorded as fullmouth, whereas it should be four-tooth.]

The experimental animals and controls were kept under the same conditions and received the same quantities of sulphur as recorded in the article mentioned above. The animals were weighed at monthly instead of at fortnightly intervals, and the results are noted in the following tables and graph:—

(2) Identified by Dr. Schonland, formerly of Rhodes University College.

TABLE 1.—GROUP A.
*Each sheep received 1.25 drachms (5 grams) of sulphur every Monday for the period 12th November, 1929,
 to 11th November, 1931.*

No.	14186.	15305.	24668.	24673.	23778.
Age.	Full-mouth.	Full-mouth.	Full-mouth.	Full-mouth.	Six-tooth.
Sex.	Ewe.	Ewe.	Wether.	Wether.	Wether.
Date of Weighing.	Weight.*	Increase.	Weight.	Increase.	Weight.
					Increase.
1930—					
12.11 (after shearing).....	109	—	106	—	82
5.12.....	109	—	106	—	82
1931—					
5.1.....	109.5	0.5	104	—2	87
5.2.....	121.5	12.5	118	12	90.5
5.3.....	126	17	119	13	88.5
5.4.....	128.5	19.5	120	14	91.5
5.5.....	130	21	122.5	16.5	98.5
5.6.....	135	26	124	18	107
5.7.....	143	34	130	24	111.5
5.8.....	142	33	129	23	111.5
5.9.....	139	30	128	22	114
5.10.....	138.5	29.5	130.5	24.5	116
5.11.....	144.5	35.5	135	29	120
Date of Shearing.....	11.11.31	11.11.31	11.11.31	—	11.11.31
Weight of 1-year old clip.....	12	9.5	10.5	—	12
Average weight of clip.....			11		

* All weights are given in pounds.

TABLE 2.—GROUP B.
Each sheep received 1.25 drachms (5 grams) of sulphur every Monday, Wednesday and Friday for the period 12th November, 1929, to 11th November, 1931.

No.	24221.	9128.	18558.	24519.	24379.	
Age.	Full-mouth.	Full-mouth.	Full-mouth.	Full-mouth.	Full-mouth.	
Sex.	Ewe.	Ewe.	Wether.	Wether.	Wether.	
Date of Weighing.	Weight.*	Increase.	Weight.	Increase.	Weight.	Increase.
1930—						
12.11 (after shearing).....	92	—	108	—	118	Died on the 10. 10. 30,
5.12.....	92	—	108	—	118	and was referred to
1931—						in the previous
5.1.....	92.5	0.5	108	—	116	report.
5.2.....	98	6	107	1	123	
5.3.....	105	13	116	2	128.5	5
5.4.....	109	17	119	3.5	130	10.5
5.5.....	110	18	122	6	134	12
5.6.....	114	22	130	9.5	139	16
5.7.....	118	26	136	14	139	21
5.8.....	122	30	134	20.5	139	21
5.9.....	120.5	28.5	133.5	22	132	14
5.10.....	122	30	132	22	131.5	13.5
5.11.....	124	32	133	23	136	18
				30.5	140	22
Date of shearing.....	11.11.31		11.11.31		11.11.31	—
Weight of 1-year old clip.....	11.5	10.5	11	14		—
Average weight of clip.....			11.625			

* All weights are given in pounds.

TABLE 3.—GROUP C.
Each sheep received 1.25 drachms (5 grains) of sulphur every day 'except Sundays' for the period 12th November, 1929, to 11th November, 1931.

No.	21643.	28549.	10947.	23683.	24420.	
Age.	Full-mouth.	Full-mouth.	Full-mouth.	Full-mouth.	Full-mouth.	
Sex.	Ewe.	Ewe.	Wether.	Wether.	Wether.	
Date of Weighing.	Weight.*	Increase.	Weight.	Increase.	Weight.	Increase.
1930—						
12.11 (after shearing).....	115	—	120	—	107	—
5.12.....	115	—	129	—	107	—
1931—						
5.1.....	115	—	121.5	1.5	110	3
5.2.....	121	6	127	7	125	18
5.3.....	124.5	9.5	125	5	124.5	17.5
5.4.....	130	15	127	7	127	20
5.5.....	127	12	126	6	129	22
5.6.....	134	19	130	10	134	27
5.7.....	140	25	135	15	143	36
5.8.....	139	24	135	15	142	35
5.9.....	134.5	19.5	135	15	142	35
5.10.....	142	27	141	21	148	41
5.11.....	146.5	31.5	143	23	153	46
Date of shearing.....	11.11.31		11.11.31		11.11.31	
Weight of 1-year old clip.....	12		7		14.5	
Average weight of clip.....			11.375			

* All weights are given in pounds.

TABLE 4.—GROUP D.

Controls—running under same conditions as Groups A, B and C but receiving no sulphur.

No.	17876.	7647.	22986.	24733.	24418.	
Age.	Full-mouth.	Full-mouth.	Six-tooth.	Six-tooth.	Six-tooth.	
Sex.	Ewe.	Ewe.	Wether.	Wether.	Wether.	
Date of Weighing.	Weight.*	Increase.	Weight.	Increase.	Weight.	Increase.
1930—						
12.11 (after shearing).....	118	—	110	—	105	—
5.12.....	118	—	110	—	105	—
1931—						
5.1.....	118	—	110	—	100	—5
5.2.....	121.5	3.5	119	9	102	—3
5.3.....	121	3	119	9	100.5	—4.5
5.4.....	123.5	5.5	120.5	10.5	113	8
5.5.....	133	15	124	14	119	14
5.6.....	135	17	128	18	103	15
5.7.....	143	25	129	19	104	16
5.8.....	135	17	130	20	108	20
5.9.....	140.5	22.5	130	20	109	21
5.10.....	140	22	128	18	111	23
5.11.....	140	22	128	18	114	26
11.11.31					116	11
Date of shearing.....	11.11.31	11.11.31	11.11.31	11.11.31	11.11.31	11.11.31
Weight of 1-year old clip.....	9	7.5	8.5	6	11	11
Average weight of clip.....			8.4			

* All weights are given in pounds.

TABLE 5 (*Extracted from Tables 1 to 4*).
COMPARATIVE TABLE.

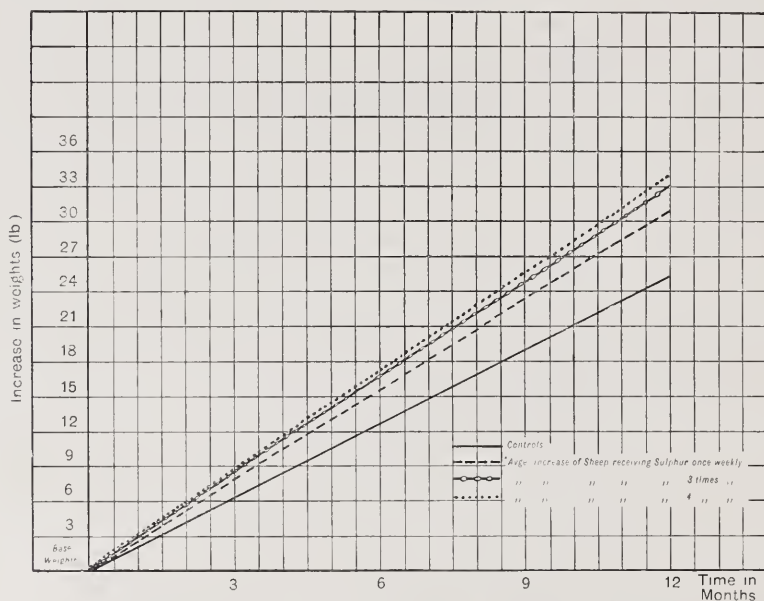
D.O.B. No.	Weight* of Sheep after shearing on 11.11.29.	Increase in Weight during 1st 3 Months of Expt.		Average Increase in Weight during 1st 3 Months of Expt.		Increase in Weight during 1st 6 Months of Expt.		Average Increase in Weight during 1st 6 Months of Expt.		Increase in Weight during 1st 9 Months of Expt.		Average Increase in Weight during 1st 9 Months of Expt.		Increase in Weight during 1st 12 Months of Expt.		Average Increase in Weight during 1st 12 Months of Expt.		Average Wool Clip each Year.	
		1930.	1931.	1930.	1931.	1930.	1931.	1930.	1931.	1930.	1931.	1930.	1931.	1930.	1931.	1930.	1931.	1930.	1931.
14186 15305 15306 24673 24550	95.5 87.5 80.5 85.5 86	18.5 11.5 11.5 14 14	12.5 12.5 12.5 4 —	— — — 14 —	— — — — —	31 22 22 9 —	21 19.5 19.5 — —	— — — 21.3 —	— — — 14.7 —	31 28.5 23.5 21.5 Died	33 30 23 23 —	— — — 27 —	— — — 28.6 —	— — — 35.5 26	— — — 32.6 —	— — — 33.5 —	— — — 10.25 11	— — — — —	— — — — —
24221 19228 24379 24379 24379	69 97.5 93 93 88.5	15 10 14.5 16.5 16.5	6 — 5 Died Died	— — — — —	— — — — —	23.5 14 31.5 35 23.5	18 14 9.5 16 —	— — 23 — —	— — 14.4 — —	30.5 14.5 42.5 33.5 33.5	30 26 22 14 —	— — 31.3 — —	— — 23 — —	37 23 56.5 43.5 40	32 25 30.5 22 —	— — 40 — —	— — — — —	— — — — —	— — — — —
21643 23406 10047 24383 24420	66 88.5 112 81.5 81.5	23.5 26.5 14.5 28 21	6 Died — 18 5.5	— — — — —	— — — — —	40.5 36.5 18.5 36.5 27.5	12 6 22 Died —	— — 31.9 — —	— — 13.3 — —	50 21 49 34 —	24 15 35 — —	— — 38.5 — —	— — 24.6 — —	— — 25 54 30	60.5 23 56.5 46 —	31.5 — 42.4 — —	— — 33.5 — —	— — 12.25 11.375 —	— — — — —
17876 7647 22986 24733 24418	116 100.5 55 90.5 74	6.5 4 6 15.5 15	3.5 9 6 — 7	— — — — —	— — — — —	14.5 15 15 32 20	15 14 15 14 12	— — 18.7 — —	— — 14 — —	18.5 15 25 37 25.5	17 20 20 16 21	— — 24.2 — —	— — 18.8 — —	— — 40.5 35 32	— — 28.2 — —	— — 25.2 — —	— — 9.3 8.4 —	— — — — —	— — — — —

* All weights are given in pounds.

Sheep 24550 (Table 1) and 23406 (Table 3) which died on the 30.9.30 and 15.7.30 respectively, were replaced by full-grown sheep 23778 and 28540. The two latter sheep have not been included in Table 5, as their inclusion would have given rise to a greater average increase in weight in those particular groups. It will be noticed from Table 5 that the average increase in weight during the first year of dosage with sulphur was strikingly more than that during the second year, except in the case of Group A.

It might be mentioned that during the course of twelve months sheep 23778 and 28540, which received sulphur once and six times weekly respectively, increased 38 lb. and 49 lb. in weight, whereas the average increase in weight of the five control sheep over the same period was 25.2 lb.

From Tables 1, 2, 3 and 4 it is evident that during the first month after shearing neither the experimental animals nor the controls gained in weight, while during the following month some gained, some lost and others neither gained nor lost.



Illustrating the effects of Sulphur on Merino Sheep.

From Table 5 it is evident that results were similar, though not identical to those obtained in the previous year. In the previous year the average increase in weight of the experimental groups of sheep was in direct relation to the amount of sulphur the animals received, while in the following year the gain in weight of the group that received least sulphur (1.5 drachms), was equal to the group that received most sulphur (7.5 drachms) and higher than the group which received 3.75 drachms sulphur weekly.

These results are quite conceivable when it is considered that only a comparatively small percentage of the quantity of sulphur taken in by the animals is absorbed.

In Groups B, C and D the average gain in weight during the year was lower than that recorded for the previous year, whereas in Group A it was higher.

The average gain in weight for all the groups during the first three, six and nine months of the second year was much less than that of the previous year.

The average wool-yield over a period of twelve months of Group D (Controls) was 8.4 lb., while the sulphur groups A, B and C yielded 11 lb., 11.625 lb. and 11.375 lb. of wool respectively.

From Table 5 it will be noticed that the wool-yield of Group A is higher than for the previous year, while in Groups B, C and D the reverse is the case.

(B) THE EFFECTS OF SULPHUR ON WORM INFECTIONS.

Before the commencement of dosing with sulphur all the experimental and control sheep were dosed with Government Wire-worm Remedy. The conditions for re-infection with worms were most unfavourable, as the drinking water was supplied in a trough and the surface of the enclosure in which the sheep were running was covered with fairly coarse gravel, so as to avoid the formation of pools of water after rains.

At the discontinuation of this experiment, Dr. Mönnig, Head of the Department of Parasitology, kindly conducted the necessary test with these sheep in order to determine whether they harboured any worms and the results of this test are incorporated in Table 6.

TABLE 6.

D.O.B. No. of Sheep.	Quantity of Sulphur Received.	Period of Dosage.	<i>O.C.</i>	<i>H.C.</i>	<i>Trich.</i>	<i>S. pap.</i>	Degree of Worm Infection.
14186	1.25 drachms once weekly	2 years	—	—	—	109	Medium.
15305			—	7	3	90	"
24668		1 year	—	—	10	90	"
23778			—	10	40	50	"
24221	1.25 drachms three times weekly	2 years	—	—	—	100	Weak.
9128			—	—	—	100	"
18558			—	—	—	100	Medium.
24519			—	20	50	30	Weak.
21643	1.25 drachms six times weekly	2 years	—	—	—	100	Weak.
10047			—	—	15	85	"
23683		1½ year	—	5	70	25	"
28540			—	—	—	100	Medium.
17876	Controls (no sulphur)	—	—	—	25	75	Weak.
7647		—	—	6	60	34	Medium.
22986		—	—	—	6	94	Weak.
24733		—	—	20	10	70	Medium.
24418		—	—	7	16	77	Strong.

O.C. = *Oesophagostomum columbianum*.

H.C. = *Haemonchus contortus*.

Trich. = *Trichostrongylus*.

S. pap. = *Strongyloides papillosus*.

From this table it would appear that the controls were more heavily infested with worms than the three groups which had received sulphur. The group that had received sulphur weekly showed stronger cultures than the two groups which had received sulphur three and six times weekly respectively.

As another *sulphur* experiment with Merino wethers is about to be commenced it is hoped that more definite information in regard to the effects of continuous dosage with sulphur on worms will be obtained.

(C) THE OCCURRENCE OF URINARY CALCULI IN THE EXPERIMENTAL SHEEP.

The deaths, which occurred in the experimental sheep, are as follows:—

TABLE 7.

D.O.B. No. of Sheep.	Group.	Weekly Dose of Sulphur (in drachms).	Date of Commencement of Dosing with Sulphur.	Date of Death.	Period of Dosage with Sulphur up to Date of Death.	Cause of Death.
		drms.			Days.	
24550 (wether)	A.	1.25	13.11.29	30.9.30	321	Jaagsiekte.
24673 (wether)	A.	1.25	13.11.29	2.6.31	445	Urinary calculi.
24379 (wether)	B.	3.75	13.11.29	10.10.30	330	Urinary calculi.
23406 (wether)	C.	7.5	13.11.29	15.7.30	243	Killed (urinary calculi).
24420 (wether)	C.	7.5	13.11.29	15.5.31	557	?

Sheep 24550, 24379 and 23406 (all wethers) have been referred to in the previous article.

Sheep 24673 (wether) was found lying down on 2.6.31 and breathing heavily. The pulse was accelerated and weak and there was pronounced salivation. The conjunctival and visible mucous membranes were intensely cyanotic and the animal was in distress. Death supervened at 12 noon on the same day. On post-mortem there was pronounced hyperaemia of the lungs and numerous calculi were causing complete obstruction of the urethra.

Sheep 24420 (wether) was found dead on the morning of the 15.5.31. The carcase was in a state of advanced decomposition and only hyperaemia and oedema of the lungs were noticed.

Out of fifteen experimental animals one died from "Jaagsiekte", two succumbed and one had to be killed as a result of urinary calculi and one died from an unknown cause. Of these fifteen animals eight were wethers and the remaining number ewes. The mortality occurred among the wethers only.

(D) THE RESISTANCE OF THE SULPHUR-DOSED SHEEP TO POTASSIUM CYANIDE POISONING.

The M.L.D. of potassium cyanide for full-grown merino sheep was found to be approximately 0.006 gm. per kilogram body-weight.

Two sheep out of each group which had received sulphur for a period of two years were selected for this test. The last dose of sulphur was administered on the day prior to the determination of the resistance of these animals to potassium cyanide.

The results are recorded in Table 8.

TABLE 8.
RESISTANCE OF SULPHUR-DOSED SHEEP TO POTASSIUM CYANIDE.

D.O.B. No. of Sheep.	Weight.	Previous Treatment.	Amount of Kcn. per kg. Body- weight.	Total Kcn. per Sheep.	M.L.D. per kg. Body- weight.	Result.
24608	Kg. 55	Received 5 gms. of sulphur once weekly for a period of two years	Grams. 0.006	Grams. 0.33	Grams. —	Very slight transient laboured respiration. Re- covered within $\frac{1}{2}$ hour.
23778	48	" "	0.012	0.576	—	Symptoms of pronounced laboured respiration within 1 minute and death within $\frac{1}{2}$ hour after dosage.
24221	50	Received 5 gms. of sulphur three times weekly for a period of two years	0.006	0.3	—	Fairly pronounced laboured respiration which disappeared within $\frac{1}{2}$ hour.
9128	55	" "	0.012	0.66	0.006	Symptoms of pronounced laboured respiration within 1 minute and death within 1 hour after dosage.
21613	60	Received 5 gms. of sulphur six times weekly for a period of two years	0.006	0.36	—	Very slight transient laboured respiration. Re- covered within $\frac{1}{2}$ hour.
28540	53	" "	0.012	0.636	—	Symptoms of severe laboured respiration within 1 minute, and death within $1\frac{1}{2}$ hours after dosage.
32313	30	Controls (— no sulphur).....	0.005	0.15	—	Symptoms of severe laboured respiration within 2 minutes after dosage. Completely paralysed within 16 minutes, no cornea reflex; gasping for breath. Recovered within 8 hours after dosage.
20778	47	" "	0.01	0.47	—	Symptoms of pronounced laboured respiration; within 1 minute. Trembling; convulsions; completely paralysed within 10 minutes. Died within 1 hour after dosage.

The three sheep (24668, 24221 and 21643) which received the M.L.D. of potassium cyanide developed very slight symptoms of poisoning while the three animals (23778, 9128 and 28540) which received twice the M.L.D. died.

The control, which received 0.005 gm. potassium cyanide per Kg. body-weight almost died, while the second control succumbed to 0.01 gm. potassium cyanide per Kg. body-weight.

It would appear from the above table that the sheep which had received sulphur for a period of two years were more resistant to potassium cyanide than the controls.

III. DISCUSSION.

(A) THE EFFECT OF SULPHUR ON THE BODY-WEIGHT AND WOOL-YIELD OF MERINO SHEEP.

Merino wethers and ewes have received sulphur at the rate of 1.25 drachms once, three times and six times weekly for a period of two years with striking beneficial results in regard to their wool-yield and increase in weight as compared with the control sheep running under identical conditions, but receiving no sulphur.

The question of the formation of urinary calculi in wethers will be referred to later on.

It is of interest to note that at the end of the second year the average yearly increase in weight of the group of sheep receiving sulphur weekly was equal to that of the group receiving six times the amount of sulphur, while at the end of the previous year there was a striking difference in the yearly average increase in weight of these two groups. This equality in the average increase of weight at the end of the second year was due to the decreased average increase in weight of the group that received sulphur six times weekly, which was bound to occur as the better the condition of the animals the less the increase in weight will be.

The average increase in weight of the group receiving sulphur three times weekly was 27.4 lb. as compared to 40 lb. during the previous year.

There was an appreciable drop in the average wool-yield of the control group and the groups receiving sulphur three times and six times weekly, respectively, as compared with the yields of the previous year, while the average yield of the group receiving sulphur weekly showed an average increase of .75 lb. as compared with the yield of the previous year.

(B) THE EFFECTS OF SULPHUR ON WORM-INFECTIONS.

Judging from the results of tests (egg-counts and cultures) obtained after the sheep had been receiving sulphur for two years it would appear that the controls harboured more worms in the intestines than the groups of sulphur-treated sheep. The sheep which had received sulphur weekly appeared to be slightly more heavily infested than the groups which had been dosed three and six times weekly respectively. The constant presence of sulphur in the gastrointestinal tract appears to have had a very slight detrimental effect on the worms mentioned in the above table.

(C) THE OCCURRENCE OF URINARY CALCULI IN THE EXPERIMENTAL SHEEP.

Of eight wethers dosed with sulphur three succumbed to the effects of urinary calculi. The percentage affected was 37.5, which is very high. It is noteworthy that the more sulphur the animal received the sooner it was affected by urinary calculi, the three cases occurring respectively 445, 330 and 243 days after the commencement of the experiment. One animal out of each of the three groups was affected.

The fact that three out of eight experimental wethers receiving sulphur succumbed to the effects of urinary calculi seems to indicate that the sulphur played a part directly or indirectly in the formation of these calculi. The calculi contained a certain amount of sulphur but this does not imply that the sulphur caused their formation. At Onderstepoort urinary calculi in sheep are of fairly common occurrence and before any definite conclusions as to whether sulphur plays, a part in the formation of urinary calculi can be drawn experiments should be conducted on a larger scale than has been the case in the above experiment.

Pontus, Carr and Doyle [*Jnl. of Agric. Res. (U.S.)* No. 42, 1931, pp. 433-446] have found that in sheep some feeds produced a urine containing 6 to 8 per cent. of solids and that the urine varied from slightly acid to strongly alkaline according to the diet supplied to the animals. The calculi were composed of calcium carbonate, calcium and aluminium phosphate, aluminium silicate, kidney tissues, urates, epithelium etc. From the above it would appear that the diet plays a most important part in the production of urinary calculi. An experiment to determine whether sulphur stimulates the formation of urinary calculi in sheep is about to be commenced.

(D) THE RESISTANCE OF THE SULPHUR-DOSSED SHEEP TO POTASSIUM CYANIDE.

In the 15th Report of the Director of Veterinary Services it was reported that favourable results in regard to sulphur as a preventive for the prussic acid form of "Geilsiekte" had been obtained. (Recent Investigations into the Toxicity of known and unknown Poisonous Plants). Subsequently this experiment was repeated on rabbits, which were dosed with sulphur and their resistance to potassium cyanide tested. The results of this experiment are published elsewhere in this report.

As all the sulphur-treated sheep and rabbits showed a certain degree of resistance to hydrocyanic acid poisoning, it was decided to repeat the tolerance test with groups of sheep that had received varying amounts of sulphur throughout two years. The results were similar to those obtained in previous experiments on sheep and rabbits, namely, the sulphur-treated sheep showed a fairly highly developed tolerance to potassium cyanide in comparison with the controls.

It must be admitted that these tests were very severe as the potassium cyanide was given to the animals in a 1 per cent. solution per stomach-tube, thus allowing for large amounts of prussic acid to be evolved within a very short time and hence leaving very little time for excretion or combination with such sulphur compounds as might have been present in the system.

It is to be expected that much more favourable results with sulphur as a preventive for prussic acid poisoning will be obtained under field conditions, where the animals ingest plants containing prussic acid at a comparatively slow rate thus allowing time for excretion.

Another fact that rendered the last test a most severe one is that the sulphur-dosed sheep were in an extremely good condition, thus having a large amount of fat in the body. As the dose of potassium cyanide was calculated per kilogram body-weight, it will be realised that the sulphur-dosed sheep received much higher doses of potassium cyanide than the controls, which were not in such an excellent condition. In regard to this test it is therefore obvious that the excellent condition of the sulphur-treated sheep was to their disadvantage, as the large amount of fat tissue raised the dose of potassium cyanide and did not assist in the excretion of the prussic acid.

CONCLUSIONS.

(a) Merino wethers and ewes, which had received sulphur over a period of two years, showed a much greater increase in body-weight and wool-yield than the control animals.

(b) The controls showed a higher degree of worm-infection than the sulphur-treated sheep.

(c) 37.5 per cent. of the sulphur-dosed wethers succumbed to the effects of urinary calculi, whereas the two control wethers are apparently still in good health.

(d) The sulphur-treated sheep showed a fair degree of resistance to potassium cyanide.

Studies in Mineral Metabolism XVIII. Phosphorus in the Nutrition of Sheep. (Final Report.)

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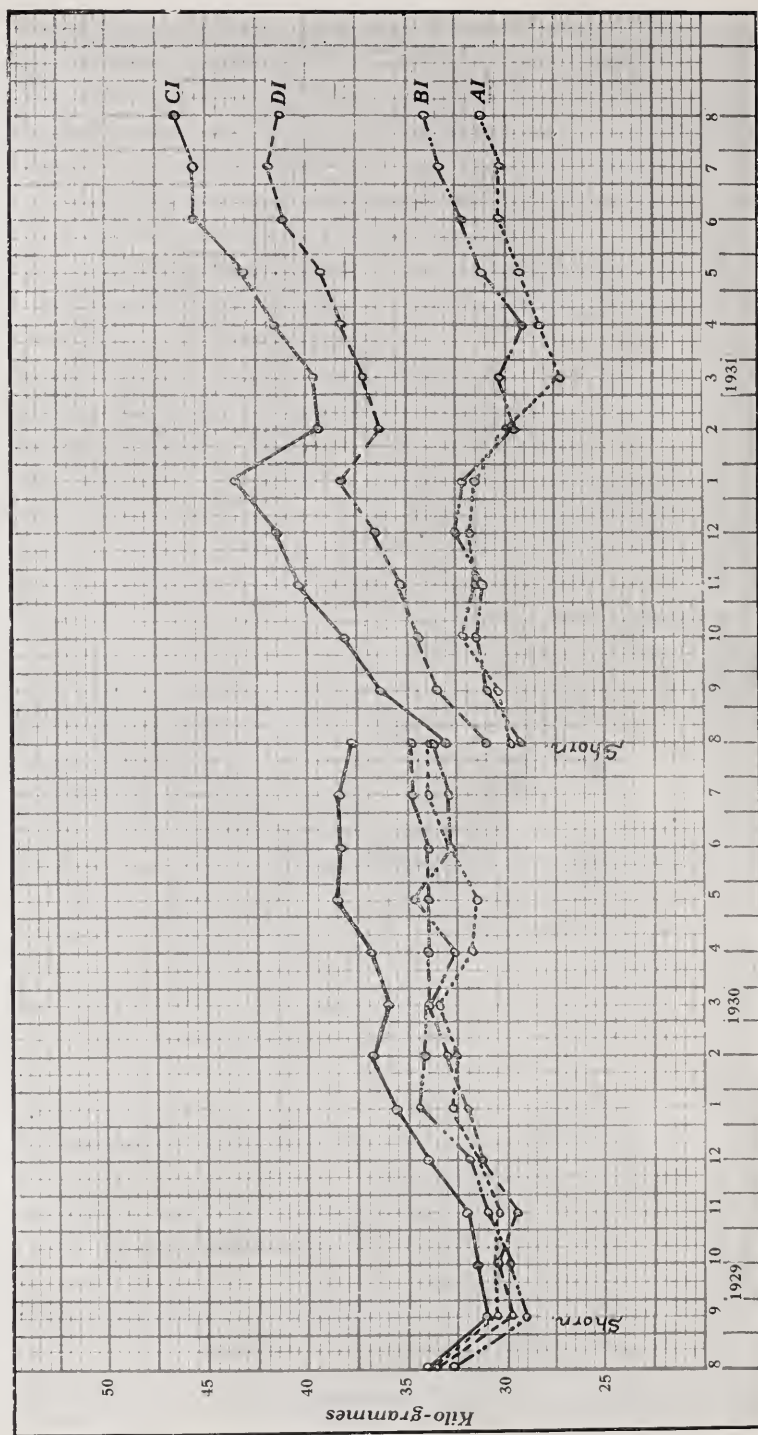
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THE investigation showing the effects of different amounts of Disodium phosphate on the food consumption, weights and inorganic phosphorus in the blood of four groups of Merino ewes, has been concluded and is finally dealt with in the present paper. Previous detailed reports upon the work appeared in the 16th and 17th Reports of the Director of Veterinary Services and Animal Industry. All the conditions, as explained in the previous reports, with regard to feeding, care and management, have been strictly adhered to throughout the two years that the trial lasted. A short synopsis of the groupings is repeated here:—

- I. Group A₁ (5 sheep) receive a ration as low as possible in phosphorus, which amounts to a daily intake of .47 gram.
- II. Group B₁ (5 sheep) receive a ration whose phosphorus content is equal to the daily intake of sheep on poor quality pasture which is common in phosphorus-deficient areas in South Africa. The amount present is .73 gram.
- III. Group C₁ (5 sheep) receive a ration whose phosphorus content is equal to the daily intake of sheep on good quality pasture. This amounts approximately to 1.53 grams.
- IV. Group D₁ (5 sheep). The phosphorus content of the ration of these sheep is in excess of their requirements, the daily amount being 2.92 grams.
- V. Group D₂ (5 sheep) consume the equivalent of .84 gram calcium in their ration daily, which is a seventh of what these animals would consume on natural pasture of .7 per cent. CaO.

A complete analysis of the wool findings for the whole experimental period is reported on in another article in this report.

Fig. 1.—Weights of Sheep.



Months.

EXPERIMENTAL WORK.

The unpublished data collected in the last eight-month experimental period very substantially corroborate what has previously been illustrated. In order, therefore, to avoid too much repetition, charts are given here showing the final results for the two-year period:—

Figure I. Weights of Sheep.

Figure II. Individual Average Weights of Sheep.

Figure III. Food Consumption.

Figure IV. Individual Average Food Consumption.

Figure V. Inorganic Phosphorus in the Blood.

Figure VI. Individual Average Inorganic Phosphorus in the Blood.

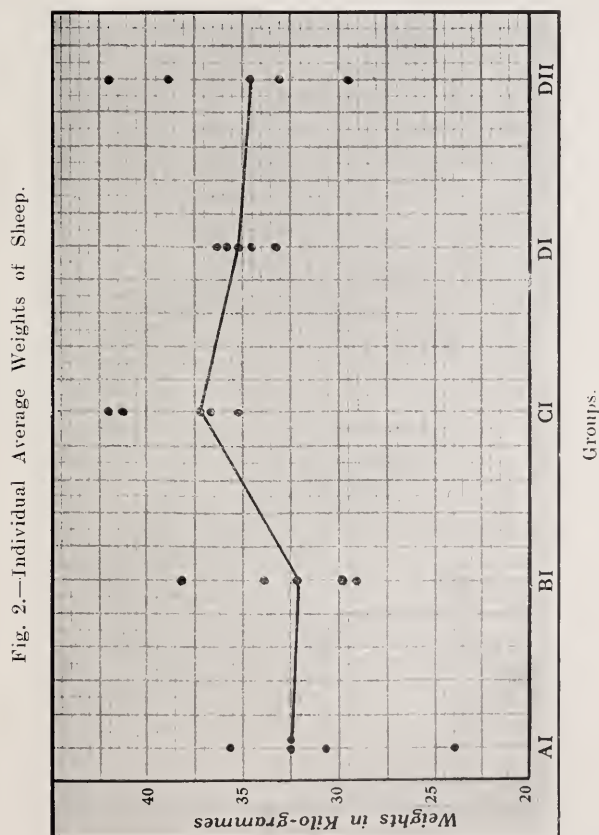


Fig. 3.—Food Consumption.

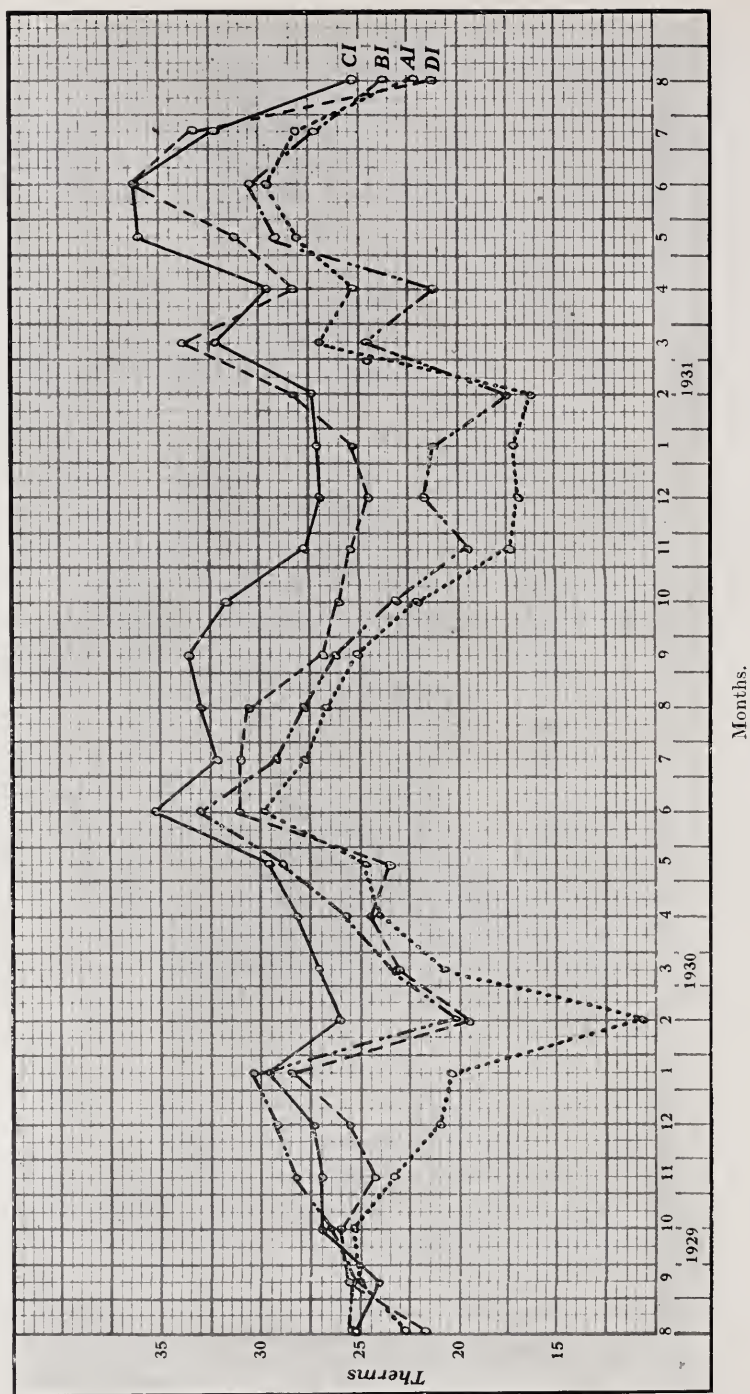


Fig. 4.—Individual Average Food Consumption.

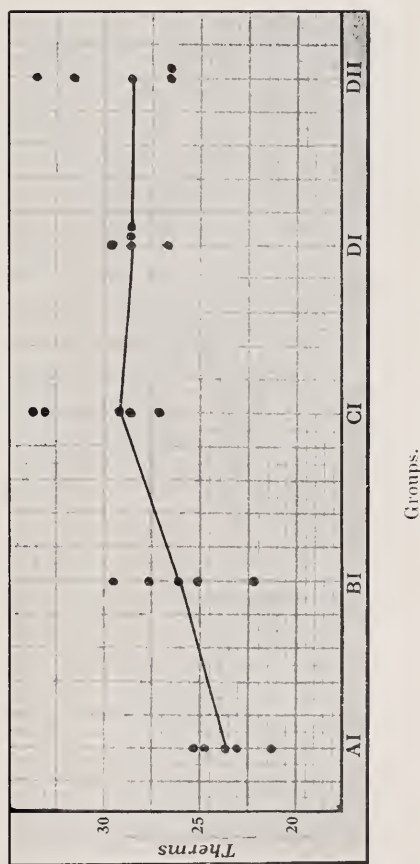
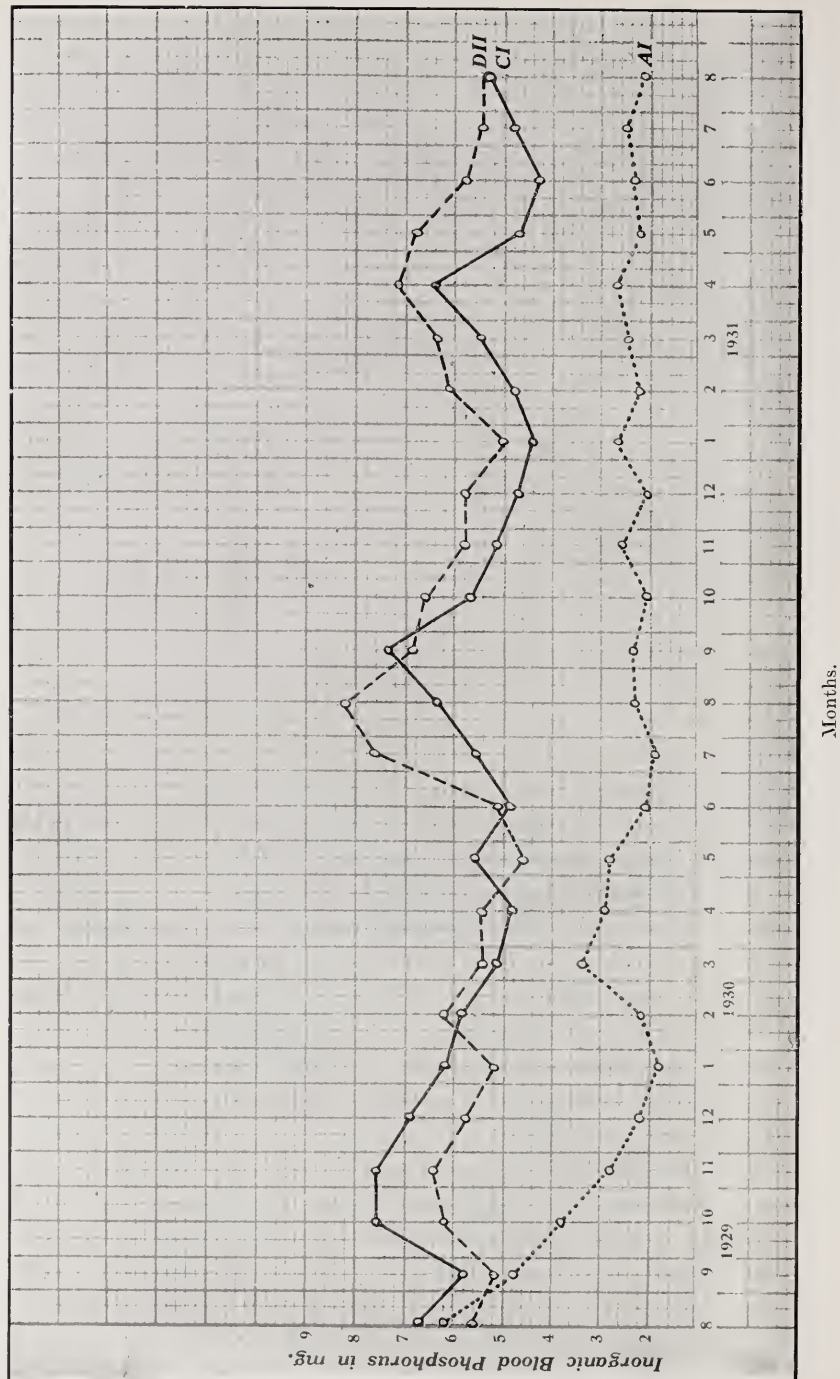
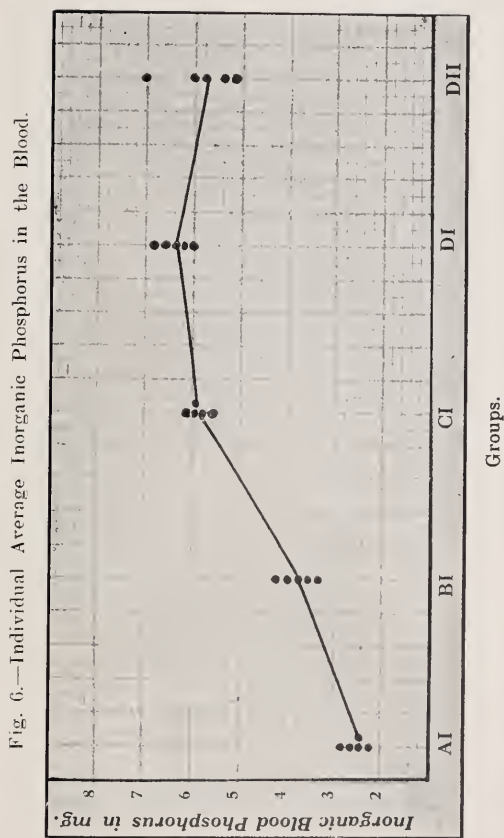


Fig. 5.—Inorganic Phosphorus in the Blood.





DISCUSSION.

Group C₁ showed greater gains in weight from the beginning of the experiment and remained the best group throughout. It is worthy of note that C₁ weighed on an average 15 Kg. per sheep more than the lightest group at the conclusion of the experiment. In food consumption also, C₁ showed the keenest appetite, while the inorganic phosphorus of the blood fluctuated but never dropped to the level of phosphorus deficiency as shown by A₁ and B₁. It is rather surprising that group D₁ did not show greater gain in weight. Its weight increase became definitely superior to that of groups A₁, and B₁, only during the latter 12 months of the experiment. On the whole it did well but it cannot be said that the phosphorus which D₁ received in excess of that of group C₁ was beneficial. In fact it seems practically certain that the optimum amount of phosphorus for sheep is nearer the intake of group C₁ than of D₁. Figures II and IV corroborate this view. The inorganic phosphorus of D₁ is definitely higher than that of C₁, as would be anticipated from the respective phosphorus intakes. Groups A₁ and B₁ are in every way the poorest groups and are readily distinguishable from the others. There is not much to choose between them, although B₁ was undoubtedly gradually gaining on A₁ during the last year of the experiment. If a glance be taken at the lambing chart in Table I it will be noticed that two lambs were reared in B₁ in 1930, whereas none in group A₁. This must have been an additional setback to the former group and probably explains its similarity to the latter, especially during the earlier part of the experiment, as shown in Figures I, II and III. Figures V and VI show the difference in the phosphorus intake of the two groups, or rather how well this difference is reflected in the inorganic phosphorus content of the blood.

The fluctuations of food consumption corresponds fairly closely with those of inorganic phosphorus in the blood. Figure V shows the difference between the inorganic phosphorus in the blood of phosphorus-deficient animals as represented by groups A₁ and B₁, very clearly when compared with their more fortunate sisters in groups C₁ and D₁. Figure VI represents the same thing more clearly, each dot being the average value for inorganic phosphorus for the total experimental period, viz., 24 months. It is worthy of note that the values for each group of sheep lie closely together and that the groups are easily distinguishable from one another on the basis of the phosphorus content of the blood which coincides, as could be anticipated, with the grouping according to the phosphorus content of the ration. Blood analysis undoubtedly plays an important part in studies of phosphorus deficiency, and is already of great assistance in this country with regard to an investigation of the geographical distribution of aphosphorosis.

In a general way the order of the curves giving food consumption corresponds closely with that of the weight curves, the poorer groups A₁ and B₁ having eaten much less than C₁ and D₁. In the earlier reports this was interpreted as evidence for smaller appetites of phosphorus-deficient animals. The ultimate result, as Figures II and IV show, is, that the groups A₁ and B₁ were consuming only

TABLE 1.—LAMBING CHART FOR 1930 AND 1931.

1930.						1931.					
D.O.B. Nos.	Groups	Gesta- tion Period, Days.	Birth Weight, lb.	Sex.	Final Weight, lb.	Remarks.	Gesta- tion Period, Days.	Birth Weight, lb.	Sex.	Final Weight, lb.	Remarks.
26153.....	A ₁	—	—	—	—	Ewe not served.	153	4·7	—	7·6	Normal lamb.
23965.....	A ₁	—	—	—	—	No lamb.	151	6·3	—	11·6	Normal lamb.
23149.....	A ₁	—	—	—	—	Ewe not served.	150	5·3	—	9·9	Normal lamb.
24007.....	A ₁	—	—	—	—	Ewe not served.	153	5·3	—	—	Lamb died day of birth.
23968.....	A ₁	—	—	—	—	No lamb.	151	—	—	—	Lamb born dead.
Averages.....		—	—	—	—		152	5·4	—	9·7	
23974.....	B ₁	150	5·8	—	11·0	Normal lamb.	—	—	—	—	No lamb.
23975.....	B ₁	152	5·4	—	10·0	Normal lamb.	152	8·7	♂	15·5	Normal lamb.
26151.....	B ₁	—	—	—	—	Ewe not served.	—	—	—	—	No lamb.
26152.....	B ₁	—	—	—	—	Ewe not served.	150	4·9	—	7·6	Normal lamb.
23978.....	B ₁	—	—	—	—	No lamb.	—	—	—	—	No lamb.
Averages.....		151	5·6	♀	10·5		151	6·8	—	11·5	
26159.....	C ₁	—	—	—	—	Ewe not served.	143	5·8	♂	12·9	Normal lamb.
23995.....	C ₁	150	9·0	—	14·9	Normal lamb.	152	6·4	♂	—	Lamb died aged 14 days.
23986.....	C ₁	148	6·2	—	14·2	Normal lamb.	148	7·5	♂	13·1	Normal lamb.
24002.....	C ₁	—	—	—	—	Ewe not served.	150	6·4	♀	6·7	Lamb normal, never
23988.....	C ₁	—	—	—	—	No lamb.	159	8·0	♀	—	able to walk.
Averages.....		149	7·6	—	14·5		150	6·8	♀	10·9	Lamb died aged 1 day.

TABLE 1.—LAMMING CHART FOR 1930 AND 1931—(continued).

1930.						1931.					
D.O.B. Nos.	Groups	Gestation Period. Days.	Birth Weight. lb.	Sex.	Final Weight. lb.	Remarks.	Gestation Period. Days.	Birth Weight. lb.	Sex.	Final Weight. lb.	Remarks.
26156.....	D ₁	—	—	—	—	Ewe not served.	151	4·5	♀ ♂	7·7	Normal lamb.
23995.....	D ₁	—	—	—	—	No lamb.	152	5·8	—	—	Died aged 2 days.
23996.....	D ₁	—	—	—	—	No lamb.	151	8·4	—	14·5	Normal lamb.
23997.....	D ₁	—	—	—	—	No lamb.	149	5·1	—	—	Died 1 day after birth.
23998.....	D ₁	—	6·3	♀	—	Died aged 8 days.	—	—	—	—	No lamb.
Averages.....		—	6·3	—	—		151	5·9	♀	11·1	
2 999.....	D ₂	—	—	♀ ♂	—	No lamb.	148	—	♂	—	Died day of birth.
24000.....	D ₂	147	5·7	—	6·8	Normal lamb.	147	5·6	♀ ♂	11·4	Normal lamb.
26158.....	D ₂	—	—	—	—	Ewe not served.	—	—	—	—	No lamb.
24001.....	D ₂	—	7·3	—	9·0	Normal lamb.	153	5·0	♀ ♂	11·0	Normal lamb.
24002.....	D ₂	150	5·3	♂	10·1	Normal lamb.	157	8·0	—	—	Died day after birth.
24003.....	D ₂	—	—	—	—		—	—	—	—	
Averages.....		148	6·1	—	9·0		151	6·2	—	11·2	

a fraction of the food eaten by C_1 and D_1 , but it does not follow at that stage as a matter of course that the decrease is directly due to the phosphorus deficiency of the former pair of groups. Aphosphorosis leads to poorer development and stunted growth so that after some time such animals would have a smaller capacity than heavier fully developed ones. Smaller food requirements would then be a natural consequence of prolonged aphosphorosis rather than an immediate result. Figure III shows that during the first year of the experiment, B_1 ate fairly well in spite of its phosphorus deficiency, although A_1 on less phosphorus than B_1 showed a poor appetite practically from the start of the experiment. Otto (1931) found that the same animal (bovine) ate better when receiving a supplement of sodium phosphate than on the phosphorus-deficient basal ration alone. During the 21 days' experimental period of phosphorus sufficiency the ox in question would invariably consume every particle of food, while portions of the same ration were left daily during the following period when the phosphorus supplement was withheld. The palatability of the food may be ruled out as a cause for better food consumption in the sheep experiment as well as in the case of the bovine referred to above, for the same basal ration was given while the phosphate was dosed daily.

Summarizing the results for the whole period the fact that phosphorus deficiency affects growth in sheep very adversely stands out clearly. That such deficiency can be diagnosed from blood analysis is another important conclusion.

The lambing chart in Table I gives the data for reproduction for the two seasons 1930 and 1931.

The results are disappointing and throw very little light upon the effect of phosphorus deficiency on reproduction in sheep. It seems that group A_1 with its extreme phosphorus deficiency bred as well as group C_1 in 1931, in spite of the poorer condition and general backwardness of the former group. Lambing in 1930 was practically a failure, as several sheep were substituted for those that had died as a result of dipping after the outbreak of scab and as conception was poor on the whole. These substituted ewes were not served by the ram owing to the lateness of the season and still further decreased the percentage that actually gave birth to lambs in 1930.

THE EFFECT OF LOW CALCIUM.

Curves are presented for the food consumption, weight increase and inorganic phosphorus in the blood of 3 groups of sheep, viz., A_1 , C_1 and D_2 . A_1 and C_1 represent phosphorus deficiency and sufficiency respectively, while D_2 received the same basal ration as A_1 and C_1 and the same quantity of phosphate as C_1 , while the calcium was withdrawn from the supplement which left the total intake of calcium very low for this group. Further details of this part of the experiment were given in the last report.

Figure VII. Food Consumption.

Figure VIII. Weights of Sheep.

Figure IX. Inorganic Phosphorus in mgm. per 100 c.c. Blood.

Fig. 7.—Food Consumption.

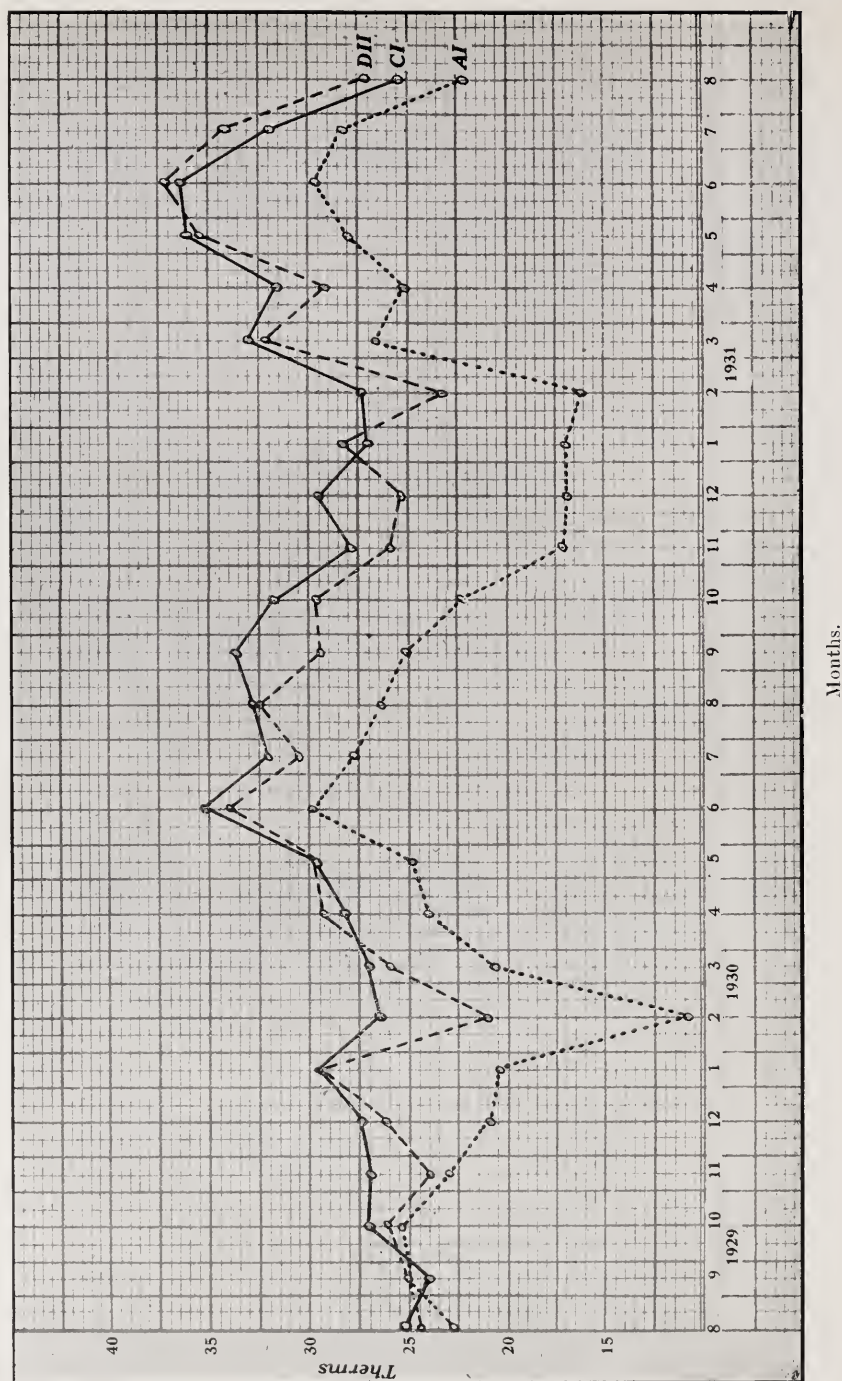


Fig. 8.—Weights of Sheep

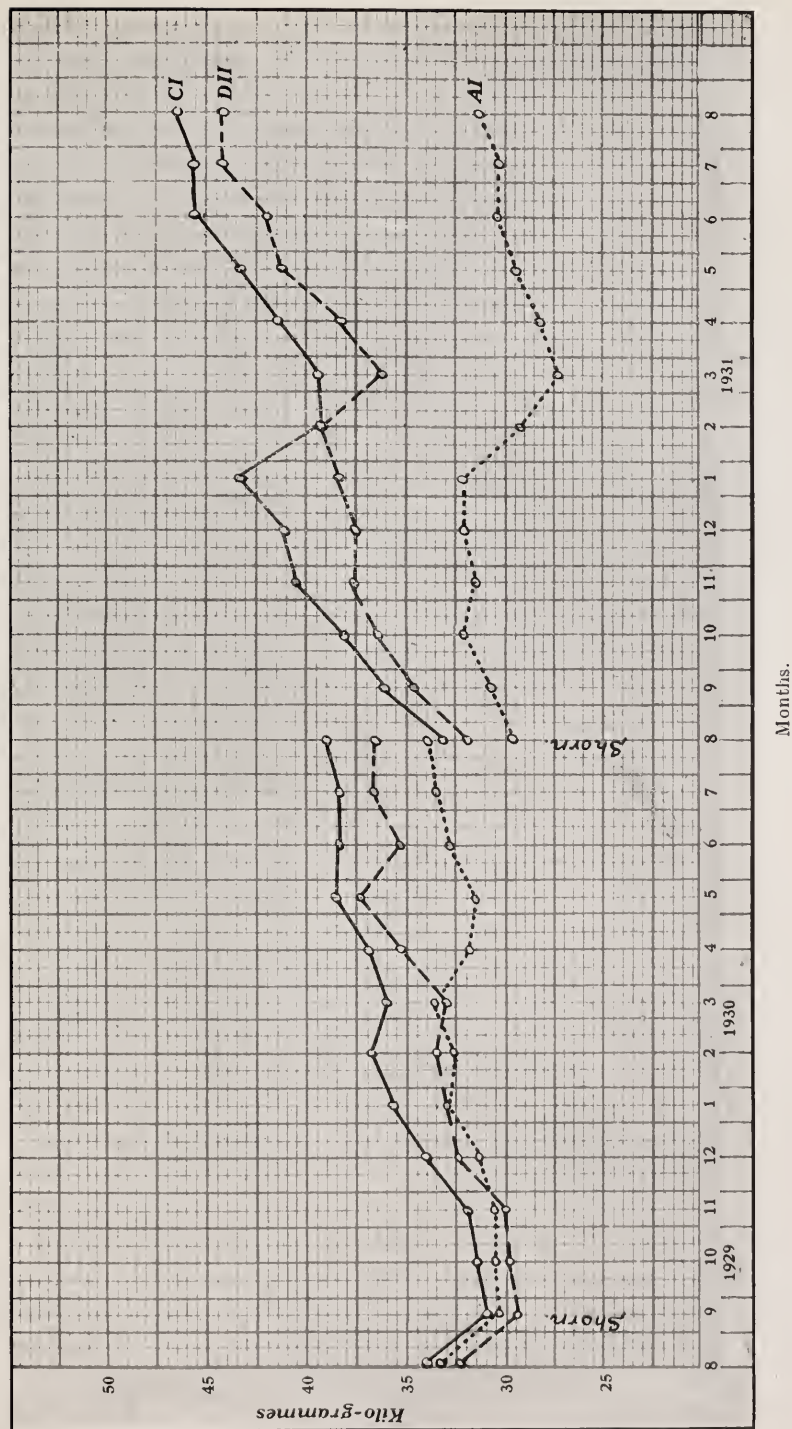
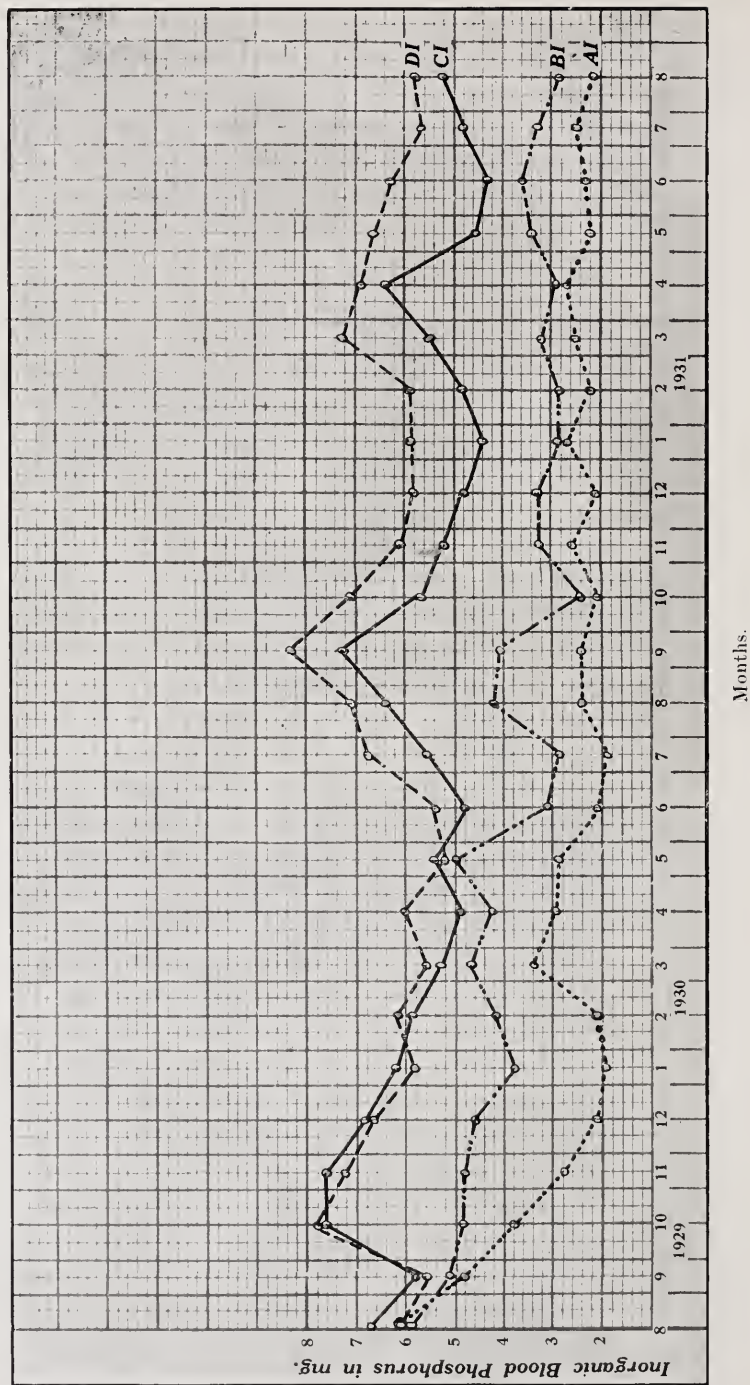


Fig. 9.—Inorganic Phosphorus in mgm. per 100 c.c. Blood.



In general the comments made in the last report still hold good. D_2 was definitely superior to A_1 and not far behind C_1 in weight increase and food consumption. As the lambing chart in Table 1 indicates group D_2 produced more lambs for the two seasons than any of the other groups except C_1 . It is not at all clear from the results that D_2 suffered from any nutritional deficiency when compared with the best group C_1 . However, the fact remains that the sheep on the calcium low ration (D_2) constantly showed depraved appetites and often ingested quantities of earth. In view of the fact that these sheep have done extraordinarily well in spite of having been on a diet extremely low in calcium it is difficult to judge what part the soil calcium played. During the second lambing season with the extra drain of lactation it cannot be said that these ewes showed a greater setback than those of group C_1 for instance, while it seems unlikely that the low Ca in the ration could have supplied the demands of the animal for this element. Nevertheless, until earth-eating has been overcome no importance can be attached to the results obtained from D_2 . This group of sheep have now been placed on concrete floors and will be kept in the experiment with the controls for at least another lambing season.

CONCLUDING REMARKS.

The practical value of the results of this investigation of the rôle of phosphorus in the nutrition of sheep is obvious. South African grasses from the Bechuanaland area containing approximately 25 per cent. P_2O_5 for the greater part of the year—a figure obtained for pastures in many other phosphorus-deficient areas in the Union—do not provide sufficient phosphorus for the animal's needs and must produce under-development, stunted growth, and poor condition, as that shown in group B_1 . Reproduction may be affected in the long run, although it cannot be said that either of the groups on low phosphorus, viz., A_1 and B_1 , was inferior in regard to reproduction to C_1 receiving an adequate amount of phosphorus. It is true that the latter group produced two lambs more during the two lambing seasons than A_1 , but it is doubtful whether the difference was sufficient to warrant more than a warning that phosphorus deficiency may lead to poor reproduction. Even then, such a conclusion seems a natural inference when considering that well-developed ewes in prime condition, such as those in group C_1 , compare very favourably with those in A_1 and B_1 , many of which were hardly able to walk properly on account of their poor condition. The very fact, however, that group C_1 receiving sufficient phosphorus in its ration gained about 30 lb. per sheep during the course of the experiment, whereas group B_1 , on the identical ration, except that its supplement of phosphate was just half of that of C_1 , and identical with the intake of sheep on South African pastures, neither gained nor lost in weight, proves beyond doubt that the deficiency of phosphorus had a very considerable retarding effect, and spells no good for the sheep farmer in phosphorus-deficient areas of the Union. In cattle, phosphorus deficiency leads to styfsiekte and depraved appetite, which usually results in the ingestion of carcass debris which may be infected with a type of pathogenic organism (*Parabotulius bovis*) causing Lamsiekte and ultimately death. Hence the cattle industry was threatened with extinction until methods had been adopted for

making good the phosphorus deficiency by feeding phosphates and other products containing phosphorus. The sheep farmer is not quite in the same plight, for only isolated cases of lambsiekte occur and he is able to carry on in spite of the phosphorus deficiency from which his sheep may be suffering. He is not forced to feed phosphates, yet, as in the case of cattle, he may vastly improve the condition of his flock and at all events increase its potentialities for production, be it mutton or wool, by making good the phosphorus deficiency of the pasture. An experiment has just been started under conditions of practical farming based upon the findings of this investigation. The area is notoriously deficient in phosphorus, although recognized sheep country. Animals will be given a phosphate supplement and for the rest, will be grazed on the veld, which will have to provide sufficient food for the control group.

In conclusion, the practical value of blood analysis for studying aphosphorosis may again be emphasized. In most arid and semi-arid areas where the growing period of the vegetation is short phosphorus deficiency is most probably a factor to contend with and blood analysis would supply the information with very little inconvenience to both the animals and the investigator.

SUMMARY.

1. The final report of the experiment on the effect of phosphorus upon the nutrition of sheep is presented.

2. Previous conclusions have been corroborated.

3. The group of sheep receiving the equivalent in phosphorus as sodium phosphate of that contained in 2 lb. of good English hay made the best gains in weight, and were in excellent condition at the end of the experiment, weighing about 50 Kgs. each. The animals on the same ration without the supplement of sodium phosphate never showed any appreciable gain in weight during the 24 months of the experiment and ended up with a smaller average weight than at the beginning of the experiment, viz., 31.5 Kg.

4. The phosphorus intake of group B₁ was equivalent to that of sheep on Bechuanaland pasture which is abundant in phosphorus-deficient areas in the Union. There is no doubt that this group of sheep suffered severe setbacks from phosphorus deficiency. Its final weight was slightly less than its initial weight.

5. The group receiving excess phosphorus grew well on the whole, but ended up with an average individual weight of 5 Kg. less than that of group C₁. Both groups were in prime condition at the conclusion of the experiment, and it can therefore only be said that excess phosphate in the ration apparently did not produce lasting detrimental results. During the first year of the experiment, D₁ remained practically on a par with the phosphorus-deficient groups A₁ and B₁ and seemed to have been at a disadvantage when compared with C₁.

6. No conclusion can be drawn from the results showing the effect of low calcium on the growth of sheep.

7. The inorganic phosphorus content of the blood was determined at regular intervals for the 24 months that the experiment lasted, and corroborated earlier data that low phosphorus in the ration was reflected as low inorganic phosphorus in the blood.

S. Reproduction was apparently unaffected by the phosphorus content of the ration.

REFERENCES.

- DU TOIT, P. J., MALAN, A. I., AND ROSSOUW, S. D. (1930). Studies in Mineral Metabolism XII. 16th Rept. Dir. Vet. Ser. and Anim. Indust. of S.A., pp. 313-326.
- DU TOIT, P. J., MALAN, A. I., AND GROENEWALD, J. W. (1931). Studies in Mineral Metabolism XVII. 17th Rept. Dir. Vet. Ser. and Anim. Indust. of S.A., pp. 454-472.
- OTTO, J. S. (1932). Studies in Mineral Metabolism XXII. Phosphorus, Calcium, and Protein. This Report.

APPENDIX.

TABLE II.—WEIGHTS OF SHEEP IN KILOGRAMS.

D.O.B.	1931.							
	Jan.	Feb.	March.	April.	May.	June.	July.	Aug.
A.I.	23965	34.9	28.5	27.2	29.0	30.8	30.8	28.1
	24007	37.1	39.9	34.1	34.1	34.9	36.7	37.1
	23968	33.6	33.6	29.9	30.8	33.1	34.4	35.8
	26153	23.1	19.9	18.6	18.1	21.3	23.1	25.3
	26149	32.2	26.3	28.1	28.5	29.0	28.1	28.5
	Total.	160.9	148.2	137.9	140.5	149.1	153.1	152.6
	Average.	32.2	29.6	27.6	28.1	29.8	30.6	30.5
B.I.	23974	25.3	24.9	24.0	23.1	24.5	24.9	25.8
	23975	36.2	28.5	29.9	28.5	32.2	34.4	34.4
	23978	39.9	38.1	38.5	38.5	39.9	41.2	43.5
	26154	31.2	33.1	33.6	32.2	33.1	34.0	34.4
	26152	30.8	25.3	26.7	25.5	27.6	29.0	28.5
	Total.	163.4	149.9	152.7	147.8	157.3	163.5	166.6
	Average.	32.7	29.9	30.5	29.6	31.5	32.7	33.3
C.I.	23985	43.5	38.5	39.9	39.9	41.7	43.5	43.5
	23986	47.5	41.7	42.1	43.5	46.2	48.9	49.8
	24008	39.9	34.9	36.2	38.1	41.2	42.1	42.1
	23988	45.3	47.5	42.6	44.8	47.5	51.4	50.4
	26159	41.7	35.3	38.1	39.4	41.7	43.5	47.5
	Total.	217.9	197.9	198.9	205.7	218.3	229.4	229.4
	Average.	43.6	39.6	39.8	41.1	43.7	45.9	45.9
D.I.	23995	42.1	43.0	39.4	40.3	43.9	45.3	43.5
	23996	39.9	34.1	33.6	34.4	37.1	38.5	41.2
	23997	35.3	36.2	38.5	39.4	37.6	37.6	40.8
	23998	34.1	34.9	38.1	39.4	39.9	43.5	47.1
	26156	39.4	36.2	36.7	37.6	38.5	40.8	41.2
	Total.	190.8	184.4	186.3	191.1	197.0	205.7	213.8
	Average.	38.2	36.9	37.3	38.2	39.4	41.1	42.8
D.II	23999	45.3	48.0	40.8	43.9	48.0	50.9	51.3
	24000	45.3	42.1	41.7	43.0	45.3	44.8	47.1
	24002	31.7	28.5	27.6	31.2	32.6	35.3	36.7
	24003	37.1	41.2	34.4	38.5	39.4	42.6	45.3
	26158	35.3	37.1	38.1	37.6	41.7	41.2	43.9
	Total.	194.7	196.9	182.6	194.2	207.0	214.8	224.3
	Average.	38.9	39.4	36.5	38.8	41.4	42.9	44.8

TABLE III.—FOOD CONSUMPTION IN KILOGRAMS, ENERGY IN THERMS.

	January.		February.		March.		April.		May.		June.		July.		August.	
	F	H	F	H	F	H	F	H	F	H	F	H	F	H	F	H
A.	6766	5570	7364	5420	12569	5930	9589	5780	9698	6050	10136	6020	4728	5870	9484	5460
	26140.	5647	4390	9579	5017	9829	5483	12898	5620	13786	6300	13650	6090	10134	5100
	23953.	6208	4380	11894	5250	11172	5920	14248	6120	12966	7270	12900	6020	9944	6010
	23968.	24007.	11813	5653	11837	5797	12428	6460	14706	7380	13558	7480	9334	6250
	23965.	12486	5960	10864	5990	10818	6260	11426	7380	10528	6500	8294	6620
	6338	4840	5724	4220	12486	5960	10864	5990	10818	6260	11426	7380	10528	6500	8294	6620
	34588	25750	33030	23290	38341	27810	53291	28970	60090	30510	63020	34650	59554	32560	47390	29440
	6917	5150	6606	4658	11668	5562	10658	5794	12018	6102	12604	6990	11910	6512	9478	5888
	14.5	2.5	13.8	2.3	24.4	2.7	22.3	2.8	25.1	2.9	26.3	3.4	24.9	3.2	19.8	2.9
	17.0	16.1	27.1	25.1	28.0	29.7	28.1	22.7
B.	6908	7540	5094	6880	6776	7320	6514	6800	9238	7610	10126	8110	9144	6850	8518	7830
	23974.	6334	4870	12316	5240	10624	5800	14728	6250	14806	6910	13328	5940	13044	5600
	23975.	9004	5040	13486	5980	10534	5370	14128	5880	14756	6770	14508	5950	10184	5850
	23978.	11278	5540	10034	4870	9486	5900	9564	5220	13056	6880	10359	6330	7444	6110
	26154.	6334	4250	10446	5640	9570	5180	11848	6230	12136	6820	12468	6100	9394	6310
	7118	5070	36800	25010	52510	30080	46446	28490	62210	32630	64880	35490	59806	31070	48584	31700
	44280	29010	7360	5182	10502	6016	9289	5698	12442	6526	12976	7098	11961	6214	9716	6340
	8856	5922	15.4	2.5	21.9	2.9	19.4	2.8	26.0	3.2	27.1	3.5	24.9	3.0	20.3	3.1
	18.5	2.9	17.9	24.8	22.2	29.2	30.6	27.9	23.4
	21.4
C.	9608	7340	11964	6650	12656	7190	10874	6360	13648	8960	14006	8110	12138	6820	8584	6150
	23985.	13404	7190	16986	7990	14214	7190	16808	8000	16706	8390	14318	7720	11034	6650
	23986.	10434	7200	14936	8730	13424	8090	17418	8970	17876	9430	15288	8950	12464	8060
	23988.	8904	6200	11846	6650	10614	6780	13688	8140	13396	8810	11938	8150	9904	6380
	24008.	12634	6370	14506	7540	11864	7590	15198	8180	14326	8710	13908	8290	10734	7420
	26159.	57520	33610	70930	38100	60990	36010	76760	39950	76310	43450	67590	39930	52720	34860
	12538	7480	11504	6722	14186	7620	12198	7202	15352	7990	15262	8690	13518	7986	10544	6972
	56760	35030	11504	6722	14186	7620	12198	7202	15352	7990	15262	8690	13518	7986	10544	6972
	11352	7186	24.0	3.3	29.6	3.7	25.5	3.5	32.1	3.9	31.9	4.2	28.2	3.9	22.0	3.4
	23.7	3.5	27.3	33.3	29.0	36.0	36.1	32.1	25.4
	27.2

TABLE III.—(continued.)

	January.		February.		March.		April.		May.		June.		July.		August.	
	F	H	F	H	F	H	F	H	F	H	F	H	F	H	F	H
D ₁ .																
26156.....	8448	8390	11134	7720	16086	8550	12404	7910	13278	8030	13526	8860	14138	8410	8134	7490
25995.....	10448	7410	11614	6770	14676	6940	13394	6950	15278	7160	14966	7670	14358	7710	9564	7370
23996.....	12548	7320	10984	6040	12886	8270	13634	7990	13758	7850	16076	8080	12438	8730	8484	7570
23997.....	9778	5740	14094	6860	15786	7670	8064	6800	11248	6610	15886	7940	12948	8340	6574	5980
23998.....	10538	8500	13184	7770	14556	9130	11844	8160	14458	8890	17556	9290	16078	9040	11944	7480
Total.....	51760	38360	61010	35160	73990	40560	59340	37810	68020	38540	78010	42440	69960	42250	44700	35890
Average.....	10352	7672	12202	7032	14798	8112	11868	7562	13604	7708	15602	8488	13992	8450	8940	7178
Therms.....	21.6	3.7	25.5	3.4	30.9	3.9	24.8	3.7	28.4	3.8	32.6	4.1	29.2	4.1	18.7	3.5
	25.3		28.9		34.8		28.5		32.2		36.7		33.3		22.2	
D ₂ .																
23999.....	11708	5760	8054	6230	15486	6420	14904	6590	16488	7240	16866	8180	15908	7570	12694	5960
24000.....	13638	6820	12614	5860	13766	7380	12184	6490	15578	7010	14286	7380	13438	7360	11374	6270
24002.....	9128	5940	8044	5720	11346	8020	10494	7540	14078	8400	16896	8800	14538	8210	11464	6600
24003.....	12218	6970	9094	6090	16186	7460	13354	6850	17198	7340	18116	8470	16658	7560	12814	6010
26158.....	13138	6460	11134	5530	12756	6280	11694	5760	13338	5960	14966	7890	13358	7270	10234	6030
Total.....	59890	31950	48940	29430	69540	35560	62630	33230	76680	35950	81130	40720	73940	37970	58580	30870
Average.....	11978	6390	9788	5886	13908	7112	12526	6646	15336	7190	16226	8144	14780	7594	11716	6174
Therms.....	25.2	3.1	20.5	2.9	29.1	3.5	26.2	3.2	32.1	3.5	33.9	3.9	30.9	3.7	24.5	3.0
	28.3		23.4		32.6		29.4		35.6		37.8		34.6		27.5	

F = Fanko, H = Hay.

PHOSPHORUS IN THE NUTRITION OF SHEEP.

TABLE IV.—INORGANIC PHOSPHORUS IN MGM. PER 100 C.C. BLOOD.

D.O.B.	1931.							
	Jan.	Feb.	March.	April.	May.	June.	July.	Aug.
A.I.								
23965	2.2	1.3	2.0	2.5	1.5	1.3	1.7	1.0
24007	1.9	2.0	2.6	2.7	3.2	2.8	2.8	4.1
23968	4.5	3.2	3.3	3.0	2.7	3.2	3.1	2.9
26153	3.1	2.5	2.3	2.6	2.1	2.3	2.8	2.7
26149	1.9	2.1	2.1	2.6	1.5	1.8	1.9	2.1
Total.	13.6	11.1	12.3	13.4	11.0	11.4	12.3	12.8
Average.	2.7	2.2	2.5	2.7	2.2	2.3	2.5	2.6
B.I.								
23974	3.0	2.3	2.3	3.4	2.0	2.2	2.5	2.1
23975	2.1	1.8	2.7	2.7	4.9	4.7	4.2	3.5
23978	3.4	3.6	4.6	3.1	4.0	4.5	3.9	2.8
26154	2.5	2.6	2.8	2.7	3.1	2.8	2.6	2.8
26152	2.5	3.4	3.8	3.1	2.9	3.6	3.5	3.6
Total.	13.5	13.7	16.2	15.0	16.9	17.8	16.7	14.8
Average.	2.7	2.7	3.2	3.0	3.4	3.6	3.3	2.9
C.I.								
23985	5.0	4.5	6.7	7.7	5.0	—	4.9	4.7
23986	5.6	5.4	6.0	5.8	4.8	5.1	5.4	6.6
24008	3.8	3.9	4.8	6.1	5.3	3.9	4.4	4.7
23988	4.9	6.2	6.5	6.9	5.5	5.3	4.9	4.6
26159	2.8	4.6	3.6	5.6	3.0	2.7	4.6	5.9
Total.	22.1	24.6	27.6	32.1	23.6	17.0	24.2	26.5
Average.	4.4	4.9	5.5	6.4	4.7	4.3	4.8	5.3
D.I.								
23995	5.6	6.6	8.6	7.1	4.6	6.5	5.9	6.6
23996	6.0	5.7	8.3	7.5	9.0	—	5.6	5.5
23997	5.4	5.4	7.1	6.8	6.1	6.0	5.7	6.2
23998	8.0	6.7	7.1	7.4	6.3	7.8	7.0	7.2
26156	4.9	5.2	5.4	6.7	7.5	4.9	4.8	3.5
Total.	29.9	29.6	36.5	35.5	33.5	25.2	29.0	29.0
Average.	5.9	5.9	7.3	7.1	6.7	6.3	5.8	5.8
D.II.								
23999	5.4	8.3	8.0	7.8	9.0	7.3	7.0	6.0
24000	5.2	5.6	5.5	6.6	5.7	3.6	4.8	4.5
24002	4.6	3.8	7.8	7.3	6.7	6.3	5.6	6.6
24003	4.6	8.6	6.8	7.8	8.5	7.1	6.2	5.8
26158	5.3	6.5	3.7	6.3	3.9	4.8	4.0	4.1
Total.	25.1	32.8	31.8	35.8	33.8	29.1	27.6	27.0
Average.	5.0	6.6	6.4	7.1	6.8	5.8	5.5	5.4

Studies in Mineral Metabolism, XIX.

Influence of Phosphorus and other Minerals on Wool Growth.

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I. INTRODUCTION.

THE results here described are based upon an experiment carried out at Onderstepoort to determine the rôle of phosphorus in the nutrition of sheep. Two Reports have already been issued (1930, 1931) and a third now appears. They are concerned with the food consumption, phosphorus and calcium content of the blood, and the sheep weights. Samples of the wool grown in the course of the experiment were sent for report to the Wool Research Laboratory, Grahamstown, and the present studies are to be considered along with those prepared by Du Toit, Malan and Groenewald.

For purposes of the experiment forty young ewes, arranged in four groups, A, B, C, D, were selected at the Grootfontein School of Agriculture. They arrived at Onderstepoort in June, 1929, and were shorn on the 13th and 14th September, when about 15 months old. The investigation was continued for two years, the actual experimental treatment beginning in August, 1929, and ending August, 1931. During the first year scab broke out, about four months after shearing, and a certain mortality occurred after dipping, necessitating the introduction of other sheep, distinguished in the tables by the letter "s" placed next to the sequence number. Hence some of the shearings in 1930 had not been grown subject to the experimental treatment throughout the year, and could not be used for full comparison with the 1929 and 1931 shearings.

The reconstituted groups were all shorn on the 12th August, 1930, the fleeces having eleven months' growth, and again on the 27th August, 1931, with slightly over twelve months' growth. The third, the 1931 shearings, were fleeces which had been grown subject to the experimental conditions throughout, and lambing took place during the first half of the period. The influence of gestation and lactation on the wool growth for the first six months is, therefore, considered, samples being taken on the 27th February from the shoulder area, cut as close to the skin as possible. Other samplings were taken from the same area at ten months after shearing, representing four months' growth, and again at twelve months, representing two months' growth.

All the sheep were fed on the same basal ration, which is just above maintenance, the full details of which were given in the first report (1930). The mineral composition of the ration differed only in its phosphorus content, and in the calcium in one sub-group, D₂. In a general way, Group A was treated to a phosphorus deficiency, Group B to low phosphorus, Group C to phosphorus sufficiency, and half of Group D to excess phosphorus, the other half to phosphorus sufficiency along with a calcium deficiency.

II. INFLUENCE OF POTASSIUM IODIDE.

In 1930 treatment with potassium iodide was introduced, and the primary Groups A, B and C were divided into the Sub-Groups A₁, A₂; B₁, B₂; C₁, C₂; with five sheep each. The feeding and phosphorus proportions remained as before, but Sub-Groups A₂, B₂ and C₂ were given daily 0.02 gram of KI in addition. According to Report 2

(1931) no appreciable difference was obtained between the two sub-groups of each primary group in 1930, but in 1931 the difference had become so marked that two such sub-groups cannot possibly any longer be regarded as one group of ten.

The separate and the combined averages of the sub-groups are given in Table 1, so far as concerns fleece weights, staple length and fibre thickness. From the table it is obvious that as far as the wool is concerned the treated and untreated sub-groups show no significant differences, the fewness of the individuals (five) in each having to be taken into account. In all except fleece weight the total results are lower under the KI treatment, which is of some interest considering the generally recognised effect on hair growth of iodine acting through the thyroid gland.

It is manifest that the treatment with the KI, under the conditions of the experiment, has not had sufficient influence on the wool to be appreciable, though suggestive in the case of the fleece weight. The differential results obtained for the separate complete groups of ten individuals can, therefore, be assigned to the influence of the varying proportions of phosphorus alone, unaffected by the division into sub-groups and the treatment with KI.

TABLE 1.

Sub-groups, five Ewes each, treated with Potassium Iodide and untreated.

Sub-Groups.	Fleece Weights.	Staple Length.	Fibre Thickness.
	Lb.	Cm.	μ .
A ₁	5.96	8.4	16.78
A ₂ , (KI).....	5.45	7.2	16.13
B ₁	6.80	8.2	17.21
B ₂ , (KI).....	6.46	8.8	16.66
C ₁	8.26	9.7	18.88
C ₂ , (KI).....	9.27	9.0	18.81
A ₁ + B ₁ + C ₁	7.01	8.8	17.62
A ₂ + B ₂ + C ₂ , (KI).....	7.56	8.3	17.20

III. INFLUENCE OF CALCIUM DEFICIENCY.

The influence of a calcium deficiency was introduced into the treatment of the five ewes in D₂, the equivalent of only 0.84 gram in their daily ration being allowed, the sufficiency proportion of phosphorus remaining the same as in Group C. In Table 2, Group C, with a sufficiency of Ca, is compared with Sub-Group D₂ having the deficiency of Ca, as regards fleece weights, staple length and fibre thickness, the condition in the preliminary period, 1929, being given as representative of the Ca influence of the Karroo.

In the 1929 shearings, as might be expected, no significant differences occur between Group C and Group D₂ in any of the three characteristics, so that any differences which appear in the 1931 shearings can be ascribed to the special Ca treatment. As regards fleece weights and fibre thickness no differences having a significant value become evident in 1931, when the groups supplied with sufficient calcium are compared with those with insufficient calcium. Staple length, however, shows a gain of 0.9, or 9.6 per cent. in favour of D₂, subject to the calcium deficiency, but is probably of little account.

The results may, therefore, be regarded as showing no special influence due to the Ca deficiency treatment introduced into the experiment. As mentioned by the writers in Report 2, it may be regarded as a remarkable fact that sheep receiving so little calcium as that given in Group D₂ should yet give weights practically the equivalent of those of sheep with a calcium sufficiency. The whole question of Ca metabolism is discussed in the reports, and it is pointed out that the animals in this group developed marked geophagia and in this way might have corrected the Ca deficiency of the ration.

TABLE 2.

Comparison of Group C with Calcium Sufficiency and Group D₂ with Calcium Deficiency.

	Groups.	1929.	1931.
Fleece Weights (lb.).....	C	8.04	8.71
	D ₂	7.46	7.94
	—	-0.58	-0.77
Staple Lengths (cm.).....	C	7.12	9.38
	D ₂	6.90	10.28
	—	-0.22	+0.90
Fibre Thickness (μ.).....	C	18.54	18.85
	D ₂	18.91	19.41
	—	+0.37	+0.56

The number of ewes in each group, namely, ten, with five in each of the Sub-Groups D₁ and D₂, was selected with the expectation that any difference in the averages of the groups, due to difference of experimental treatment, would have a significance. The differences between Group A and Group B are, however, usually found to be so small, whether as regards fleece weight, fibre thickness or staple length, that their probable errors show them to have little or no significance. Suggestive values are indicated in some cases, but the number included in the experiment is obviously too small to justify any reliance being placed upon them.

Similarly with the averages of the Groups C, D₁ and D₂, all of which are higher than those in Groups A and B. Their probable errors show that in few instances are the fleece differences sufficiently large to have any significance among themselves. If, however, we regard Groups A and B as a single group and Groups C, D₁ and D₂ as another, thereby increasing the number of individuals in each from ten to twenty, the two groups present a marked difference. The account thus tends to resolve itself into a comparison of a Group A and B with low or insufficient phosphorus, and a Group C, D₁ and D₂ with sufficient or excess phosphorus, and the results of the treatment then have a real significant value with a direct bearing on farming practice.

IV. FLEECE WEIGHTS.

All the fleeces were weighed at Onderstepoort in the unscoured condition, prior to any sorting or skirting, no equipment for precise scouring being available at the time. The weighings naturally include the weight of wool produced, and also the weight of grease and adhering foreign particles, such as sand. It may be presumed that the solid particles will be about the same in weight and character in each fleece since the sheep were maintained under the same conditions. As regards the grease weight it is highly probable that Groups C and D, in a higher condition of nutrition, will have a higher proportion of grease than those in a reduced condition, Groups A and B. The fleece weight will, therefore, vary with the scoured weight of wool and the proportion of grease, but the two cannot be compared separately in the unscoured fleece.

The results indicate, however, that a real value can be attached to the weighings of the unscoured fleeces, for they reveal no significant difference among the groups in the averages of the 1929 preliminary period, while decided differences occur in the fleeces grown under the experimental conditions. Thus Group A shows a difference of 23.9 per cent. between the 1929 and 1931 shearings, while Groups D₁ and D₂ give no significant difference between the two. Spencer, Hardy and Brandon (1928) discuss the value to be attached to unscoured as compared with scoured fleeces. They found that a close correlation (-0.283 ± 0.131) exists between the two. Furthermore, their results show that either scoured or unscoured fleece weights could be used in making comparisons with other fleece characters, such as fibre thickness, though not for staple length.

The fleece weights for the 1929, 1930, and 1931 shearings are given in Table 3. The 1929 weights represent the fleeces grown mostly at Grootfontein during the preliminary period, and are the first shearings. The averages of the five groups show only slight differences, and the probable errors indicate that no significance is to be attached to them. The co-efficients of variability of the groups also reveal that the entire shearing may be regarded as almost uniformly constituted.

TABLE 3.

Fleece Weights of Shearings 1929, 1930 and 1931 (lb.).

Group A.—Phosphorus Deficiency.						Group B.—Low Phosphorus.					
No.	1929.	No.	1930.	No.	1931.	No.	1929.	No.	1930.	No.	1931.
1	7.00	1s	8.75	1s	6.00	11	6.62	11	5.38	11	5.90
2	9.75	2s	8.00	2	6.60	12	6.81	12	4.56	12	5.40
3	7.00	3s	7.19	3s	5.00	13	7.50	13s	8.25	13s	8.00
4	7.56	4s	8.12	4s	6.00	14	7.50	14s	7.62	14s	5.50
5	8.56	5	7.31	5	6.30	15	8.19	15	8.75	15	9.20
6	8.00	6	5.56	6	—	16	8.50	16s	6.31	16s	6.30
7	7.44	7s	7.69	7s	6.50	17	8.25	17	7.75	17	6.90
8	7.19	8s	6.50	8s	4.40	18	9.12	18	7.00	18	7.50
9	7.00	9	6.25	9	—	19	7.56	19	5.69	19	5.00
10	7.00	10	5.31	10	—	20	8.00	20	7.62	20	6.60
Mean.....	7.65		7.07		5.82	Mean..	7.81		6.89		6.63
E. of M..	±0.182		±0.230		±0.194	E. of M.	±0.154		±0.275		±0.266
C. of V...	11.3%		15.2%		13.1%	C. of V.	9.3%		18.7%		18.8%

Group C.—Phosphorus Sufficiency.

No.	1929.	No.	1930.	No.	1931.
21	8.25	21s	7.12	21s	7.50
22	7.12	22	7.00	22	8.40
23	9.00	23	8.19	23	8.50
24	6.69	24s	8.12	24s	7.70
25	8.19	25	8.25	25	9.20
26	8.62	26	7.62	26	9.00
27	7.25	27	7.50	27	9.60
28	9.75	28	7.00	28	9.20
29	7.69	29	—	29	9.30
36	7.81	30	—	30	—
Mean.....	8.04		7.60		8.71
E. of M..	±0.187		±0.119		±0.089
C. of V...	10.9%		6.6%		8.0%

Group D ₁ .—Excess Phosphorus.						Group D ₂ .—Phosphorus Sufficiency. Calcium Deficiency.					
No.	1929.	No.	1930.	No.	1931.	No.	1929.	No.	1930.	No.	1931.
31	7.38	31s	8.50	31s	9.00	36	9.00	36	7.31	36	8.60
32	8.25	32	8.00	32	8.80	37	7.25	37	8.00	37	8.70
33	7.00	33	6.19	33	6.20	38	7.00	38s	8.00	38s	8.60
34	9.37	34	6.50	34	6.80	39	6.75	39	6.50	39	6.00
35	9.00	35	7.56	35	9.10	40	7.31	40	6.50	40	7.80
Mean..	8.20		7.35		7.98	Mean..	7.46		7.26		7.94
E. of M.	±0.274		±0.265		±0.370	E. of M.	±0.240		±0.202		±0.308
C. of V.	11.1%		11.9%		15.4%	C. of V.	10.6%		9.2%		12.9%

Comparison of Fleece Weights of 1929 and 1931 Shearings.

Groups. {		A.	B.	C.	D ₁ .	D ₂ .	A + B.	C + D ₁ + D ₂ .	Difference.
Fleece Weights (lb.)....	1929	7.65	7.81	8.04	8.20	7.46	7.73	7.93	n.s.
	1931	5.82	6.63	8.71	7.98	7.94	6.30	8.32	32.0 %
		23.9%	15.1%	8.3%	n.s.	n.s.	18.5%	n.s.	

Most of the 1930 shearings had been subject to the experimental treatment for the entire growing period, and the averages of the groups are all slightly lower than those of the preliminary period. No significant difference occurs between any two of the groups, but Groups A and B, together making up twenty individuals, give a slightly significant difference from the combined Groups C and D, also representing twenty sheep. This significance is better shown in the 1931 shearings. The lower and somewhat irregular weights of 1930 are presumably due to the growth being only for eleven months and the adverse conditions during the year resulting from disease, calling for re-constitution of the groups.

The 1931 shearings, of slightly over twelve months' growth, are from sheep all of which had been subject to the experimental treatment during the entire period of the growth. The averages of the fleeces are conspicuously different from those in 1930 and 1929, those of Groups A and B being lighter and those of Groups C and D heavier. The fleeces in Group A are 23.9 per cent. lighter in 1931 than in 1929, and in Group B they are 15.1 per cent. lighter, while the fleeces in Group C in 1931 are 8.3 per cent. heavier than in 1929. The difference between Group D₁ in 1931 and 1929 is not significant, nor is that in Group D₂. The fleeces in Groups A and B, grown with a deficiency of phosphorus, are together 32 per cent. lighter than those in Groups C and D having a sufficiency of phosphorus, a very decided indication of the importance of a lack or sufficiency of phosphorus on wool production generally, a result concerning which many practical observations are also available.

V. STAPLE LENGTH.

The staple length of the wool represents the wool while in its crimped grease state, and is a character of the fleece with which the farmer and woolman are concerned. In making the estimations the straight staples, as cut from a sheep in sampling, are divided into twenty or more separate parts, and each one measured against a scale, the average length being taken. The method is that followed by Spencer, Hardy and Brandon (1928) and by Burns (1931) in their studies of wool production, and the results are shown to be of value for comparative purposes.

TABLE 4.

Staple Lengths of Shearings 1929, 1930 and 1931 (cms).

Group A.—Phosphorus Deficiency.						Group B.—Low Phosphorus.					
No.	1929.	No.	1930.	No.	1931.	No.	1929.	No.	1930.	No.	1931.
1	7.4	1s	8.0	1s	8.4	11	6.8	11	7.7	11	9.3
2	9.1	2	7.9	2	9.1	12	7.1	12	7.0	12	6.9
3	7.7	3s	7.3	3s	6.7	13	8.1	13s	7.2	13s	7.0
4	7.7	4s	8.0	4s	8.9	14	7.0	14s	6.8	14s	7.0
5	6.7	5	7.2	5	9.0	15	6.9	15	8.1	15	8.7
6	6.7	6	7.4	6	—	16	7.0	16s	7.9	16s	9.3
7	7.9	7s	6.8	7s	7.0	17	7.9	17	7.8	17	9.0
8	6.1	8s	7.1	8s	7.5	18	6.9	18	7.4	18	9.1
9	6.5	9	7.1	9	—	19	8.0	19	7.2	19	8.4
10	7.2	10	7.6	10	—	20	7.5	20	8.6	20	8.0
Mean.....	7.29		7.44		8.08	Mean..	7.32		7.57		8.27
E. of M..	±0.177		±0.085		±0.235	E. of M.	±0.103		±0.112		±0.221
C. of V...	11.4%		5.4%		11.4%	C. of V.	6.6%		6.9%		12.6%

Group C.—Phosphorus Sufficiency.

No.	1929.	No.	1930.	No.	1931.
21	7.3	21s	6.7	21s	9.0
22	7.0	22	7.1	22	9.9
23	8.0	23	9.3	23	10.2
24	7.3	24s	8.5	24s	9.1
25	6.9	25	8.0	25	10.1
26	6.5	26	7.4	26	9.2
27	6.5	27	7.0	27	9.1
28	6.9	28	6.6	28	8.6
29	7.8	29	—	29	9.2
30	7.0	30	—	30	—
Mean.....	7.12		7.57		9.38
E. of M..	±0.096		±0.212		±0.117
C. of V...	6.4%		11.7%		5.5%

Group D₁.—Excess Phosphorus.Group D₂.—Phosphorus Sufficiency.
Calcium Deficiency.

No.	1929.	No.	1930.	No.	1931.	No.	1929.	No.	1930.	No.	1931.
31	7.6	31s	7.1	31s	8.4	36	6.8	36	5.8	36	10.2
32	6.6	32	8.0	32	9.3	37	8.2	37	8.5	37	10.7
33	6.2	33	7.4	33	9.3	38	6.4	38s	9.0	38s	10.2
34	7.4	34	7.3	34	8.3	39	7.2	39	7.6	39	10.3
35	8.0	35	7.1	35	10.2	40	5.9	40	6.8	40	10.0
Mean..	7.16		7.38		9.10	Mean..	6.90		7.54		10.28
E. of M.	±0.603		±0.330		±0.184	E. of M.	±0.218		±0.385		±0.070
C. of V.	2.8%		4.5%		6.7%	C. of V.	10.6%		16.9%		2.2%

Comparison of Staple Lengths of 1929 and 1931 Shearings.

Groups. {		A.	B.	C.	D ₁ .	D ₂ .	A + B.	C + D ₁ + D ₂ .	Difference.
Staple Length (cm.)..	1929	7.29	7.32	7.12	7.16	6.90	7.31	7.07	n.s.
	1931	8.08	8.27	9.38	9.10	10.28	8.18	9.54	16.6%
		n.s.	13.0%	31.7%	27.0%	49.0%	11.9%	34.9%	

Table 4 gives the staple length of the shearings for 1929, 1930 and 1931. For 1929 the averages of the five groups are seen to be much alike, the differences having no significant value. Similarly with those for 1930, no differences of any significance occur, the influence of the differential phosphorus treatment evidently being masked by the introduction of the substitutes. In the 1931 shearings all the groups are longer than in 1929, the difference in Group A being, however, not significant, while that in Group B is 13 per cent., the three others being much higher. The phosphorus sufficiency Groups C and D are 16.6 per cent. longer than the deficiency Groups A and B.

The staple lengths of 1931 are longer than those of 1929 in all five groups. But this can scarcely be regarded as representing the true conditions of growth, since the fleece weights and fibre thicknesses of Groups C and D are not greater than in 1929, and are even less in Groups A and B. The length difference is doubtless to be associated with the method of taking the samples and the longer period of growth. The 1929 samples were clipped with ordinary shears, which rarely remove the wool close to the skin, whereas the 1931 samples were clipped with fine scissors immediately next the skin. The former were first shearings and by no means would the lambs give uniform fleeces of twelve months' growth, whereas the 1931 shearings were of twelve and a half months' growth. In these the differences between the Groups A and B and the Groups C and D amount to 16.6 per cent.

VI. THICKNESS MEASUREMENTS.

The thickness measurements give the average fibre thickness throughout the whole length of the staple, thus including all the variations in growth which may occur. The sample staples are first divided into numerous strands, and a proportion taken from each series, and formed into a single bundle. This is then cut along its whole length into short fragments, and the thousands of pieces uniformly intermingled in a tube of ether, and afterwards dried and mounted in euparal, measurements of 250 of the fibres being made under a Zeiss-Hegener micro-camera. The results show a high degree of consistency, as in Table 10, where the thickness measurements of the 1931 12-month samples agree most closely with those from the four separate shorter samplings.

Fibre thickness measurements were made of the following series of samples: (*a*) The shearings during the preliminary period, 1929, before the beginning of the experimental treatment; (*b*) The 1930 shearings, grown during the group re-constitution; (*c*) The 1931 shearings grown for the first half during gestation and lactation and for the second under normal conditions; (*d*) Separate four-month and two-month samples as well as others for the whole twelve months.

TABLE 5.

Thickness Measurements of Wool Samples from Shearings 1929 and 1931 (microns).

Group A.—Phosphorus Deficiency.				Group B.—Low Phosphorus.			
No.	1929.	No.	1931.	No.	1929.	No.	1931.
1.....	17.22	1s	15.42	11.....	19.18	11	16.16
2.....	18.72	2	16.42	12.....	17.71	12	15.71
3.....	19.49	3s	15.67	13.....	20.40	13s	17.93
4.....	18.59	4s	17.75	14.....	18.29	14s	17.32
5.....	18.50	5	18.66	15.....	19.61	15	18.95
6.....	20.69	6	—	16.....	19.05	16s	16.68
7.....	19.95	7s	16.47	17.....	18.58	17	15.94
8.....	20.24	8s	15.80	18.....	18.68	18	18.23
9.....	21.43	9	—	19.....	18.84	19	16.77
10.....	21.12	10	—	20.....	16.42	20	15.68
Mean.....	19.60		16.60	Mean.....	18.68		16.94
E. of M.....	± 0.270		± 0.312	E. of M.....	± 0.219		± 0.233
C. of V.....	6.5%		7.3%	C. of V.....	5.5%		6.5%

Group C.—Phosphorus Sufficiency.

No.	1929.	No.	1931.
21.....	16.95	21s	17.50
22.....	19.11	22	20.04
23.....	20.09	23	20.10
24.....	15.63	24s	17.42
25.....	19.56	25	19.34
26.....	18.67	26	17.79
27.....	17.96	27	19.84
28.....	17.99	28	16.62
29.....	20.47	29	20.98
30.....	19.01	30	—
Mean.....	18.54		18.85
E. of M.....	± 0.297		± 0.324
C. of V.....	7.5%		7.6%

TABLE 5 (*contd.*).

Group D ₁ —Excess Phosphorus.				Group D ₃ —Phosphorus Sufficiency. Calcium Deficiency.			
No.	1929.	No.	1931.	No.	1929.	No.	1931.
31.....	16.52	31s	20.19	36.....	20.87	36	21.49
32.....	21.34	32	22.50	37.....	19.06	37	20.07
33.....	19.58	33	18.04	38.....	19.19	38s	18.72
34.....	16.77	34	16.04	39.....	17.86	39	17.64
35.....	18.33	35	18.89	40.....	17.56	40	19.13
Mean.....	18.51		19.13	Mean.....	18.91		19.41
E. of M.....	±0.542		±0.730	E. of M.....	±0.353		±0.389
C. of V.....	9.7%		12.6%	C. of V.....	6.2%		6.6%

*Comparison of Fibre Thickness Measurements of 1929 and 1931
Shearings.*

Fibre Thick- ness.....	Groups. {		A.	B.	C.	D ₁ .	D ₄ .	A. + B.	C + D ₁ + D ₄ .	Difference.
	1929	1931	19.60	18.68	18.54	18.51	18.91	19.14	18.63	n.s.
			16.60	16.94	18.85	19.13	19.41	16.80	19.07	13.5%
			15.3%	9.3%	n.s.	n.s.	n.s.	12.2%	n.s.	

1929 *Shearings*.—Table 5 gives the thickness measurements for the 1929 and 1931 shearings. In both series the co-efficient of variability of the different groups is fairly uniform. In the pre-experimental series, 1929, the average thickness of the groups reveals a close uniformity, any differences having no significant value. The individuals selected for Group A, however, give shearings on the average slightly thicker than those for the other groups, but this is found to have no biometrical significance, the groups being constituted of such small numbers.

1930 *Shearings*.—The first shearings grown entirely at Onderstepoort were taken on 11th August, 1930, and represent a little less than 11 months' growth. In many cases they showed a zone of weakness in the wool, corresponding with the period of disease, the influence on the thickness in two samples being given in the Table 6.

TABLE 6.

Thickness Measurements through Zone of Weakness in two 1930 Samples compared with those Above and Below.

Sample.	Above Weak Zone.	Through Weak Zone.	Below Weak Zone.
1.....	18.0 μ	16.5 μ	20.7 μ
2.....	17.5 μ	15.7 μ	19.0 μ

The wool became finer in the region of the weak zone, but more than fully recovered afterwards. With an irregularity of this nature not much value could be assigned detailed measurements of the samples as regards the response of the fleeces to the differential treatment, and they are, therefore, not given in Table 5. A mass estimation of each group was, however, made, and is compared with the 1929 measurements (Table 7). The averages reveal no material difference in the two series.

TABLE 7.

Mass Thickness Measurements of the 1930 and 1929 Shearings.

Group.	1930.	1929.
A.....	19.13 μ	19.60 μ
B.....	18.16 μ	18.63 μ
C.....	18.60 μ	18.54 μ
D.....	18.68 μ	18.71 μ
	18.64 μ	18.87 μ

1931 *Shearings*.—Compared with those of the preliminary, 1929, shearings the 1931 thickness measurements of the whole staple length are significantly less as regards Groups A and B (12.2 per cent.), and are practically the same as regards Groups C and D. As Tables 8 and 10 show, the reduction due to lactation had some slight influence on the thickness during the particular period, but need not be taken into account in considering the difference between the phosphorus sufficiency and deficiency groups, namely, 13.5 per cent. Under the experimental treatment the sheep fed on low or insufficient phosphorus diet have undergone a significant thinning, while those fed a diet with sufficiency of phosphorus have retained their original thickness. The slight difference in fibre thickness in the calcium sufficiency Group D₂ between the 1929 and 1931 shearings is in favour of the deficiency, but is not large enough to have any significance.

INFLUENCE OF GESTATION AND LACTATION.

Lambing took place during the months of January and February, 1931, the ewes having been served from 20th August to 7th October, 1930. All the ewes lambed except Nos. 7, 11, 13, 15, 17, 29, 35 and 38, while at different periods Nos. 5, 6, 8, 9, 10, 16, 18, and 27 aborted. In each case the live lambs were weaned 21 days after birth. Wool samples were taken on the 27th February, 1931, shortly after weaning, and represent nearly six months' growth from the previous shearing on the 11th August, 1930. In most of the samples a distinct break or zone of weakness occurred below the middle of the staple. Comparisons of the time of lambing and the zone of weakness showed that the latter corresponds with the lambing period, the part above to that of gestation and the part below to the short period of lactation.

The zone of weakness was cut out of each staple and separate thickness measurements made of the part above and below. Samples from sheep not lambing and not showing a weak zone were cut midway. The measurements are given in Table 8, and in most cases reveal a decided difference, the upper part corresponding with gestation, having an average of $19\cdot26\mu$ and the lower, corresponding with lactation, an average of $16\cdot86\mu$, a difference of 12·5 per cent.

TABLE 8.

Variation in Wool Thickness during Six Months of Gestation and Lactation (microns).

Group A.—Phosphorus Deficiency.			Group B.—Low Phosphorus.		
No.	Gestation.	Lactation.	No.	Gestation.	Lactation.
1s.....	16·84	14·17	11.....	17·19	16·32
2.....	17·55	16·06	12.....	16·92	13·14
3s.....	20·02	12·71	13s.....	18·11	18·23
4s.....	18·75	16·33	14s.....	19·59	16·08
5.....	20·08	17·99	15.....	19·50	19·20
6.....	—	—	16s.....	16·53	16·48
7s.....	17·40	15·42	17.....	16·96	16·20
8s.....	15·84	15·07	18.....	19·21	19·09
9.....	17·37	14·29	19.....	17·61	16·53
10.....	20·74	13·61	20.....	17·66	13·93
Mean.....	18·29	15·07	Mean.....	17·93	16·52
E. of M.....	$\pm 0\cdot357$	$\pm 0\cdot338$	E. of M.....	$\pm 0\cdot238$	$\pm 0\cdot400$
C. of V.....	8·7%	9·9%	C. of V.....	6·2%	11·4%

TABLE 8 (*contd.*).

Group C.—Phosphorus Sufficiency.		
No.	Gestation.	Lactation.
21s.....	17·64	15·74
22.....	21·28	17·93
23.....	21·11	17·13
24s.....	18·55	14·91
25.....	19·51	16·40
26.....	18·47	16·92
27.....	20·00	19·73
28.....	17·96	16·19
29.....	23·06	21·44
30.....	—	—
Mean.....	19·82	17·38
E. of M.....	$\pm 0·382$	$\pm 0·434$
C. of V.....	8·6%	11·1%

Group D ₁ .—Excess Phosphorus.			Group D ₂ .—Phosphorus Sufficiency. Calcium Deficiency.		
No.	Gestation.	Lactation.	No.	Gestation.	Lactation.
31s.....	20·90	18·51	36.....	25·04	17·31
32.....	24·03	20·15	37.....	20·92	18·87
33.....	18·10	16·47	38s.....	18·53	17·92
34.....	18·07	14·76	39.....	18·40	15·90
35.....	17·86	18·53	40.....	19·49	18·18
Mean.....	19·79	17·68	Mean.....	20·47	17·63
E. of M.....	$\pm 0·723$	$\pm 0·546$	E. of M.....	$\pm 0·739$	$\pm 0·302$
C. of V.....	12·1%	10·2%	C. of V.....	12·0%	8·6%

Summary, compared with Preliminary Period.

Group.	1929, Preliminary.	Gestation.	Lactation.
A.....	19·60	18·29	15·07
B.....	18·63	17·93	16·52
C.....	18·54	19·82	17·38
D ₁	18·51	19·79	17·68
D ₂	18·91	20·47	17·63
Mean.....	18·83	19·26	16·86

In the summary the measurements are compared with those of the shearings in 1929. The thickness during the gestation period is seen to be greater than that during the preliminary period, while

during the lactation period it is less. Groups A and B are, however, thinner than in 1929, while Groups C and D are much thicker. Also during the period of lactation the first two groups are thinner than the remaining three groups.

As regards the slightly increased thickness during gestation it is well known that, while pregnant, ewes tend to remain in a high metabolic condition, but to be reduced during lactation, unless specially well fed. The thickness differentiation between the Groups A and B and Groups C and D during both gestation and lactation, can only be ascribed to the lack of phosphorus in the former group and its sufficiency in the latter.

It is manifest that the thinning in the lower part of the staple is to be associated with the reduced skin metabolism consequent upon lambing and lactation. This is evident when a comparison is made with the two series of measurements given in Table 9, taken from the staples of the ewes without lambs, No. 7 being the only exception. The average thickness in the lower half of the staple is but slightly less than that in the upper half, that is, 17.91μ compared with 18.58μ . The ewes which aborted also show a small difference, while all those producing lambs give the significant difference shown in Table 8.

TABLE 9.
Fibre Thickness in Ewes without Lambs (microns).

No.	Upper Part.	Lower Part.
7.....	17.40	15.42
11.....	17.19	16.32
13.....	18.11	18.23
15.....	19.50	19.20
17.....	16.96	16.20
29.....	23.06	21.44
35.....	17.86	18.53
38.....	18.52	17.92
Mean.....	18.58	17.91

4-Month and 2-Month Samplings.—The thickness measurements are given in Table 10 and summarized in Table 11, and may be compared with those of Table 8. The average thickness is much above that during the lactation period, and is nearly the same as that of the gestation period. Thus it is manifest that the thinning of the fibres during the lactation period is only temporary; after weaning, the wool recovers its ordinary thickness.

TABLE 10.

Thickness Measurements of 4-Month and 2-Month 1931 Samples.
(microns.)

Group A.—Phosphorus Deficiency.			Group B.—Low Phosphorus.		
No.	4 Months.	2 Months.	No.	4 Months.	2 Months.
1.....	16.96	13.70	11.....	15.97	15.15
2.....	17.15	14.92	12.....	17.10	15.68
3.....	15.50	14.46	13.....	17.62	17.77
4.....	18.11	17.72	14.....	16.97	16.63
5.....	18.35	18.24	15.....	18.48	18.63
7.....	17.15	15.91	16.....	17.83	15.87
8.....	16.33	15.95	17.....	15.74	14.84
9.....	16.87	—	18.....	18.99	15.62
			19.....	16.72	16.21
			20.....	16.40	14.71
Mean.....	17.05	15.84	Mean.....	17.17	16.11

Group C.—Phosphorus Sufficiency.

No.	4 Months.	2 Months.
21.....	18.23	18.38
22.....	20.26	20.69
23.....	21.22	20.93
24.....	17.91	18.30
25.....	20.14	21.31
26.....	18.16	17.61
27.....	20.00	19.61
28.....	16.82	15.52
29.....	20.41	19.02
Mean.....	19.24	19.04

Group D ₁ .—Excess Phosphorus.			Group D ₂ .—Phosphorus Sufficiency, Calcium Deficiency.		
No.	4 Months.	2 Months.	No.	4 Months.	2 Months.
31.....	20.20	21.16	36.....	23.60	24.02
32.....	22.56	23.28	37.....	20.86	19.63
33.....	18.85	18.72	38.....	19.68	18.74
34.....	15.57	15.76	39.....	18.33	17.91
35.....	18.84	20.33	40.....	18.93	19.92
Mean.....	19.20	19.85	Mean.....	20.28	20.04

The 4-month samples were taken 27th June, 1931, from the same area as the 6-month samples on 27th February. The 2-month samples were taken on 27th August, again from the same area; they, therefore, represent the growth from the same follicles.

Groups A and B are practically the same for the 4-month growth and also Groups C and D₁; for the 2-month growth both A and B are finer than for the 4-month, while C remains about the same and D₁ is but slightly stronger. In both series D₂ is slightly stronger than D₁.

The 2-month samples were grown during the middle of winter, July-August, and presumably Groups A and B being in a lower nutritive condition had a greater seasonal response than Groups C and D, which are on a higher nutritive plane.

The thickness measurements of each sample has also been measured along the whole length of the staple and are given in the last column of Table 11. These may be compared with the other thickness measurements of the 6-month (gestation and lactation), 4-month and 2-month fibres, and afford a marked confirmation of the accuracy of the measurements.

TABLE 11.
Summary of Thickness Measurements, 1931 (microns).

Group.	Gestation.	Lactation.	4 Months.	2 Months.	Mean.	12 Months.
A.....	18·29	15·07	17·05	15·84	16·56	16·60
B.....	17·93	16·52	17·17	16·11	16·93	16·94
C.....	19·82	17·38	19·24	19·04	18·87	18·85
D ₁	19·79	17·68	19·20	19·85	19·13	19·13
D ₂	20·47	17·63	20·28	20·04	19·60	19·41
Mean.....	19·26	16·86	18·59	18·18	18·22	18·19
A and B deficient Phosphorus....	18·11	15·79	17·11	15·97	16·75	16·77
C, D ₁ , and D ₂ suf- ficient phosphorus....	20·03	17·56	19·57	19·64	19·13	19·06

Percentage thickness of means in favour of sufficient phosphorus = 14·21 per cent.

VII. CORRELATIONS.

During the preliminary period, before subjecting the sheep to experimental treatment, the various weighings and measurements show that the averages of the groups have no significant difference as regards fleece weight, staple length and fibre thickness. As a result, however, of the later treatment differences appear among them in all the three characteristics. It may now be determined how far the changes in one direction vary along with those of another; for example, do the fleece weights of the groups vary in a corresponding direction and to a corresponding degree when compared with the body weights, or with the fibre thicknesses, or with the staple lengths?

The co-efficient of correlation has been calculated for the six pairs of combinations which are possible from the four characters mentioned and, as seen in Table 12, all show a high degree of correlation, implying that one varies as the other and almost to the same degree. The results serve to justify such practical conclusions as that when the body weight increases so does the fleece weight, or, that as the fibres thicken the fleece weight increases and the staples are longer.

TABLE 12.

Correlations of Fleece Weights, Body Weights, Staple Lengths and Fibre Thickness, 1931.

	Fleece Weights.	Body Weights.	Staple Lengths.	Fibre Thickness.
Fleece Weights.	—	+ .9634 \pm .0219	+ .9208 \pm .0457	+ .8909 \pm .0922
Body Weights.	+ .9634 \pm .0219	—	+ .9009 \pm .0568	+ .9285 \pm .0413
Staple Length.	+ .9208 \pm .0457	+ .9009 \pm .0568	—	+ .9070 \pm .0535
Fibre Thickness	+ .8909 \pm .0922	+ .9285 \pm .0413	+ .9070 \pm .0535	—

The experiments show that sheep with a deficiency of phosphorus in their diet are lighter in weight and produce a lighter fleece, shorter in length and of finer quality, as compared with sheep fed with a sufficiency of phosphorus. The high correlation serves to establish such fundamental issues as that the wool of a sheep changes in weight, length and thickness as the body becomes heavier or lighter; as a sheep increases in body weight its fleece increases in length and thickness, as a sheep decreases in weight its fleece becomes lighter, its wool shorter and finer.

VIII. SUMMARY.

1. An experiment was undertaken at Onderstepoort to determine the influence on the body weight, reproduction and wool growth of sheep when fed on a deficiency or low phosphorus diet, compared with sheep fed on a sufficiency or excess phosphorus diet. The experiment continued for two years, and shearings were obtained in 1929, 1930, and 1931, the first one prior to the commencement of the treatment. The present paper is concerned only with the wool growth.

2. Forty selected ewes were divided into five groups, the first three of ten each and the other two of five each, and treated as follows:—Group A, with phosphorus deficiency; Group B, with low phosphorus; Group C, with phosphorus sufficiency; Group D₁, with excess phosphorus; Group D₂, with phosphorus sufficiency and calcium deficiency. Treatment with iodine was also introduced.

3. The wools have been studied as regards fleece weight, staple length and fibre thickness or quality. Prior to the commencement of the treatment the groups differed in no significant degree.

4. Under the conditions of the experiment sheep treated with potassium iodide showed no significant difference in their wool compared with untreated sheep; likewise, no difference of any significant value was obtained from the wool of the sheep treated to a calcium deficiency (D_2).

5. For final presentation of the results it is shown that Groups A and B may be regarded as a single group of twenty sheep fed with a diet deficient in phosphorus, and Groups C, D_1 and D_2 as a similar group fed with a sufficiency of phosphorus.

6. The results are as follows: (a) The fleece weights of the phosphorus sufficiency group were 32 per cent. heavier than those of the phosphorus deficiency group; (b) The staple length on the average was 16.6 per cent. longer in the phosphorus sufficiency group than in the deficiency group; (c) The fibre thickness was 13.5 per cent. more in the former than in the latter.

7. The wool growth was reduced by 12.5 per cent. in thickness during lactation as compared with gestation, the reduction in the phosphorus sufficiency group being 14.21 per cent. less than in the deficiency group. The average thickness recovered after lactation.

8. Sheep fed with what may be regarded as an excess of phosphorus (D_1) showed no advantage in wool growth compared with a sufficiency of phosphorus.

9. The experiments served to establish that sheep with a deficiency of phosphorus in their diet produce a lighter fleece, shorter in length and of finer quality, as compared with sheep fed with a sufficiency of phosphorus.

10. As sheep increase in body weight the fleece weight also increases, and the wool becomes longer and stronger; as sheep decrease in body weight the fleece becomes lighter, the wool shorter and finer.

REFERENCES.

- BURNS, ROBERT, H. (1931). "Monthly Wool Growth of Rambouillet Ewes." *The Journ. of Text. Inst.*, Vol. 12, No. 2. T98-T109.
- DU TOIT, P. J.; MALAN, A. I.; AND ROSSOUW, S. D. (1930). "Studies in Mineral Metabolism, XII. Phosphorus in the Sheep Industry." *16th Rept. of the Dir. of Vety. Serv. and A.I., Union of S.A.*, 313-326.
- DU TOIT, P. J.; MALAN, A. I.; AND GROENEWALD, J. W. (1931). "Studies in Mineral Metabolism, XVII. Phosphorus in the Nutrition of Sheep." (2nd Report.) *17th Rept. of the Dir. of Vety. Serv. and A.I., Union of S.A.*, 453-472.
- DUERDEN, J. E. (1929). "Standards of Thickness and Crimps in Merino Grease Wools." *Journ. of Text. Inst.*, Vol. 20.
- DUERDEN, J. E. (1929). "Wool Research in South Africa." *Paper No. 64, Pan-African Agricultural and Veterinary Conf., Dept. of Agric., Pretoria.*
- SPENCER, D. A.; HARDY, J. I.; AND BRANDON, MARY J. (1928). "Factors that Influence Wool Production with Range Rambouillet Sheep." *Tech. Bull. No. 85, Off. United States Dept. of Agr., Washington, D.C.*

Studies in Mineral Metabolism.—XX. Iodine in the Nutrition of Sheep.

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INTRODUCTION.

COMMERCIAL interests have taken the fullest advantage of the uncertainty which prevails as regards the rôle of iodine in the nutrition of livestock. This has led to further confusion, and it is therefore not surprising to find that, with the results of innumerable experiments tabulated, finality on the question of feeding iodine to stock has not yet been reached. It must be added, in fairness to the investigators, that experiments have been conducted for a very wide range of different conditions so that this in itself explains partly the diversity of results. There can be no doubt that in some iodine-deficient areas or where endemic goitre occurs, judicious iodine administration has given beneficial results. At all events there appears to be no doubt that harmful effects in healthy animals receiving continued small doses of iodine have not been demonstrated, provided the doses have been sufficiently small or the period of dosage not sufficiently long to produce detrimental results. It becomes obvious that this limiting factor of time and dosage is one that may vary for different conditions and may lead to dissimilar results by different workers. For instance, Orr, Crichton and Middleton's work (1929) may give the impression that 3 gm. is the maximum dose of iodine for sheep, whereas in reality this grossly exceeds the maximum dose if the experiment is carried beyond the period of duration of Orr's experiment. Other investigators have obtained bad results with smaller doses given over a longer period. Again in our own work the dose of potassium iodide, viz., 0.02 gm. daily showed practically no detrimental effect during the first year, but leaves no doubt about its results in the second season. In the opinion of the writers the duration of the experiment is a matter of the utmost importance in iodine metabolism. The second factor, viz., size of dose, is closely associated with the iodine content of the basal ration which undoubtedly shows relatively great differences for different countries with the probable result that a dose showing beneficial effects under one set of conditions may have harmful effects or at all events may show no benefit under another.

If it is accepted that the iodine requirement of animals is minute, although not necessarily less definite than that for calcium and phosphorus, it seems necessary to supply information concerning the iodine content of rations with the results of all investigations into the problem of iodine deficiency. Such information will tend to provide some common basis for workers when comparing results, although it is freely admitted that it by no means makes the results entirely comparable. For instance, it appears more than probable to the writers that other limiting factors of growth may intensify the effects, adverse or otherwise, of iodine administration to animals. This matter will be dealt with more fully further in the text, but by way of explanation it may be mentioned here that if iodine be given to a group of animals on a ration deficient in phosphorus, the iodine may have a further retarding effect, whereas, if fed to a group getting adequate phosphorus, this need not be so. In such cases knowledge of the iodine content of the ration would not necessarily lead to correct interpretation. The point, however, remains that a comparable basis is approached if full details of the composition of experimental rations are given by all investigators. In view of this, it is surprising to find that the iodine content of the rations is rarely given, especially as quite reliable methods have been evolved for determining iodine in minute quantities in organic material.

Literature.—No attempt will be made to go into the vast amount of literature on the subject of iodine metabolism. Perhaps one of the best recent reviews of one aspect of this subject is given by Carroll, Mitchell and Hunt (1930). They present a critical study of the work done on the feeding of potassium iodide to swine, while the excellent publication by Orr and Leitch (1929) on Iodine in Nutrition presents an exhaustive review of experimental work done on this problem. Carroll, Mitchell and Hunt's work demands careful attention for it reports negative results and questions the validity of most previous data. It must be mentioned that the investigators imposed special conditions in working with smaller groups, making one animal of a twin or triplet the unmedicated control and forcing all to ingest the same amount of food daily. Results which are apparently different have been obtained by other workers under different conditions, although it is interesting to note that the writers make the following statement:—

“ A critical analysis of published experiments on the effect of supplemental iodides on the growth of animals not obviously suffering from hypothyroidism, shows that the majority indicate no beneficial effect, and that the experiments which have been interpreted in a positive way, are either statistically inadequate, or have been demonstrably misinterpreted.” Whether this opinion is correct, we are unable to say, but it does strengthen our own view that, on the whole, the effects of iodine feeding have not been sufficiently spectacular to warrant other than very conservative views on its beneficial effects when it is continuously fed in small quantities in countries or regions where hypothyroidism is not prevalent. It is felt that, at the present stage, the case for or against iodine feeding must be regarded as not proven.

DESCRIPTION OF EXPERIMENT.

The details of the investigation are set forth in the second report on Phosphorus in the Nutrition of Sheep by du Toit, Malan and Groenewald, 1931. Briefly the experiment may be outlined as follows:—The effect of iodine administration has been tested on sheep at three different levels of phosphorus intake. All the sheep received the same basal ration of hay, flaked maize and blood meal. The intake of mineral constituents was the same throughout, as explained in the first report (1930), except for phosphorus, which was kept as low as possible in Group A, the idea being to produce clinical symptoms of phosphorus deficiency. The intake of phosphorus in Group B was made equivalent to that of sheep on poor quality pasture in South Africa of approximately .25 per cent. P_2O_5 and in Group C equivalent to that of sheep on good quality pasture of about .5 per cent. P_2O_5 . The sheep were kept in an open shed and fed in separate feeding boxes. Food consumption and weight increase were registered regularly. All the minerals were dosed daily except Sundays while blood analyses for Phosphorus were made at regular intervals. The sheep were allowed to exercise in small enclosures on either side of the shed and were inspected daily for disease and examined periodically for intestinal worms.

The investigation was begun with fifteen months old ewes selected by sheep and wool officers in August, 1931, and carried on for two years. Five months after the commencement of the experiment scab broke out, and as a result of dipping a certain mortality occurred, necessitating the substitution of nine new sheep selected by the same sheep and wool officers from the same original flock.

Each of the three Groups A, B and C consisted of ten individuals and the subgroups A_1 and A_2 , B_1 and B_2 , C_1 and C_2 of 5 each. Subgroups A_2 , B_2 , and C_2 received a daily dose of .02 gm. potassium iodide.

The basal ration of all the animals consisted of 300 gm. of hay low in minerals, 450 grams of flaked maize and 20 grams blood meal. A detailed analysis of the basal ration is given in the first report by du Toit, Malan and Rossouw (1930), from which it may be necessary to reproduce only the phosphorus and iodine intake of the various groups.

Group.	A_1 .	A_2 .	B_1 .	B_2 .	C_1 .	C_2 .
Phosphorus content of daily ration gm.....	.47	.47	.73	.73	1.53	1.53
*Iodine content gm.....	.001	.016	.001	.016	.001	.016

* Iodine in ration: Bloodmeal 698 γ per 100 gm.

Flaked Maize 52.5 γ per 100 gm.

Hay 236.7 γ per 100 gm.

KI supplement 15 milligrammes.

The intake of minerals except phosphorus and iodine, was as nearly the same throughout the groups as it was possible to make it; for a study of this point reference must be made to the first report mentioned above. The mineral content of the ration was calculated on the basis of the intake of sheep on pasture, i.e. about 2 lb. of dried natural herbage daily. The quantity of potassium iodide given to subgroups A₂, B₂, and C₂ was arrived at from a study of such supplements generally given. Orr *et al.* (1929) gave sheep .3 gm. potassium iodide for 62 days without any ill effects. Evvard fed pregnant ewes .003, .013 and .052 gm. potassium iodide respectively in 1925, and when the experiment was reported on in 1928, no ill effects had yet been noticed. The largest dose given by Evvard is 2½ times that given in this investigation. Tinline (1923) states that ewes were given and ate .1 to .17 grams potassium iodide per day. He recommends giving ewes .028 gm. iodine or the equivalent of .04 gm. KI daily. Golf and Birnbach (1927) reported good effects of .04 gm. KI on lambs. When the dose was increased to .18 gm. ill effects became noticeable.

It was, therefore, decided to give a daily supplement of .02 gm. KI to each ewe in the subgroups A₂, B₂, and C₂, as this appeared to be a medium dose.

EXPERIMENTAL WORK.

(1) *Increase in weight.*— Figures I, II, III, IV and V present curves of the average increase in weight of the groups with their respective controls.

The weight curves given above are interesting and rather remarkable in certain respects. It is noticeable at a glance that the subgroups A₁ and A₂ showed gradually increasing differences in weight from the other groups almost from the beginning of the experiment until at one stage as much as 10 Kg. difference was registered. This is rather remarkable when it is remembered that this difference is not due to rapid growth and consequent weight increase of one group, but to greater decrease in weight of subgroup A₂. It may be taken for granted that the extreme phosphorus deficiency affected both subgroups adversely, but the setback was undoubtedly intensified in subgroup A₂. This aspect will again be referred to when reproduction is discussed.

The same remarks apply to subgroups B₁ and B₂, although the difference between the average weights of the subgroups is not marked and may be looked upon as hardly significant without knowledge of the data presented in Table 1. There is a slight increase in weight in both subgroups when compared with their original weights.

In Figure III the effect of iodine feeding is more or less the reverse of what is seen in Figure II. Subgroup C₂ remains slightly better in condition throughout, and it must be added that, whereas subgroup A₂ was easily distinguishable from subgroup A₁ to the eye, subgroup B₂ and C₂ could not be distinguished from their corresponding subgroups.

Fig. I.

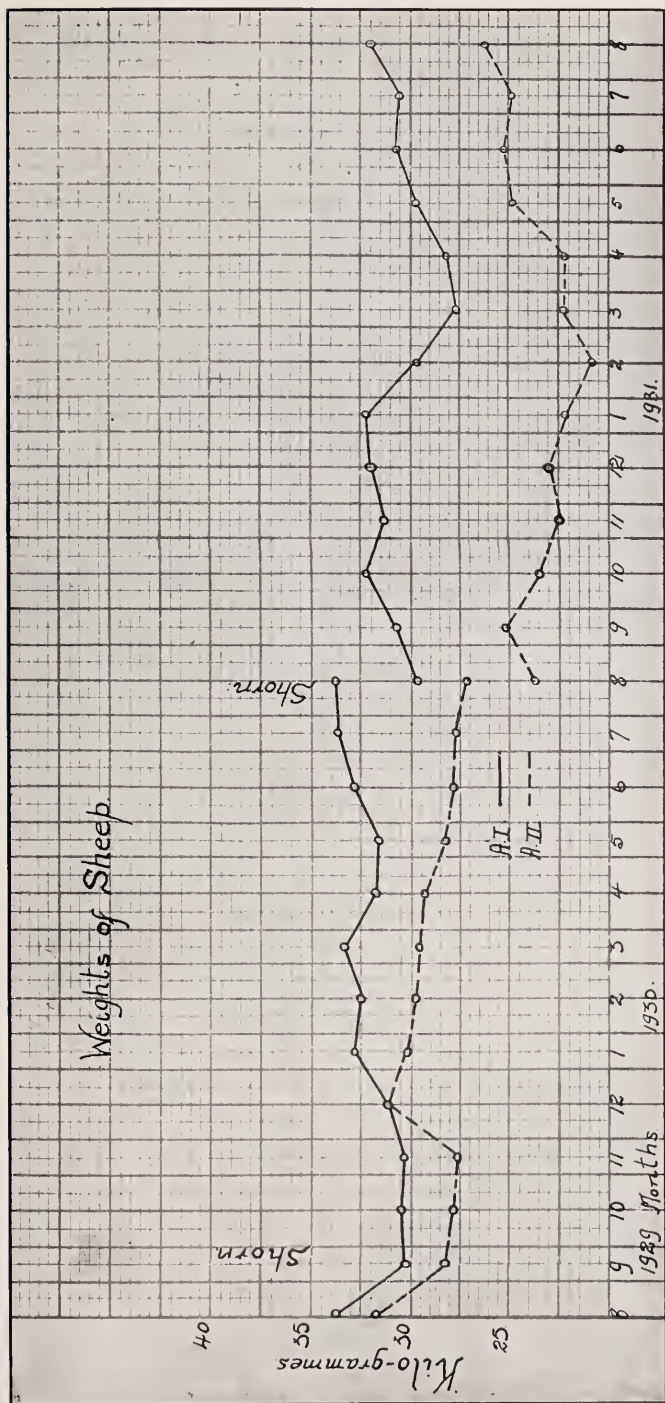


Fig. II.

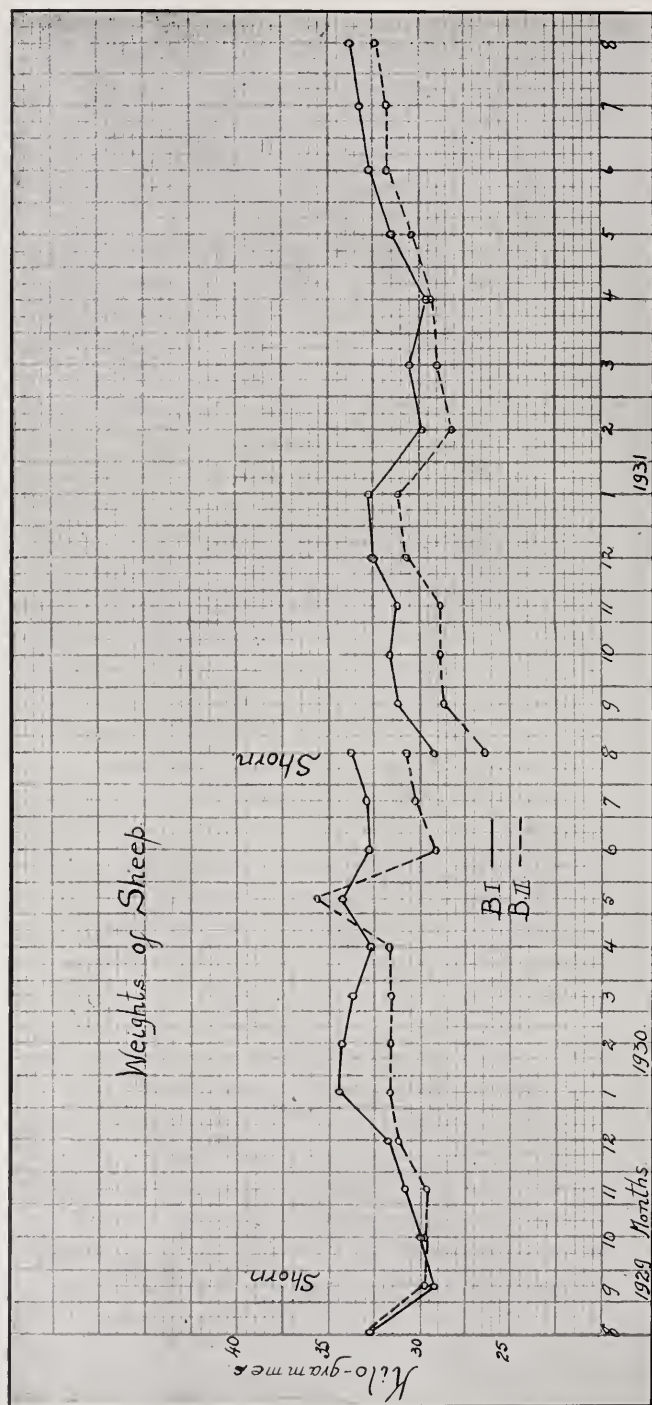


FIG. III.

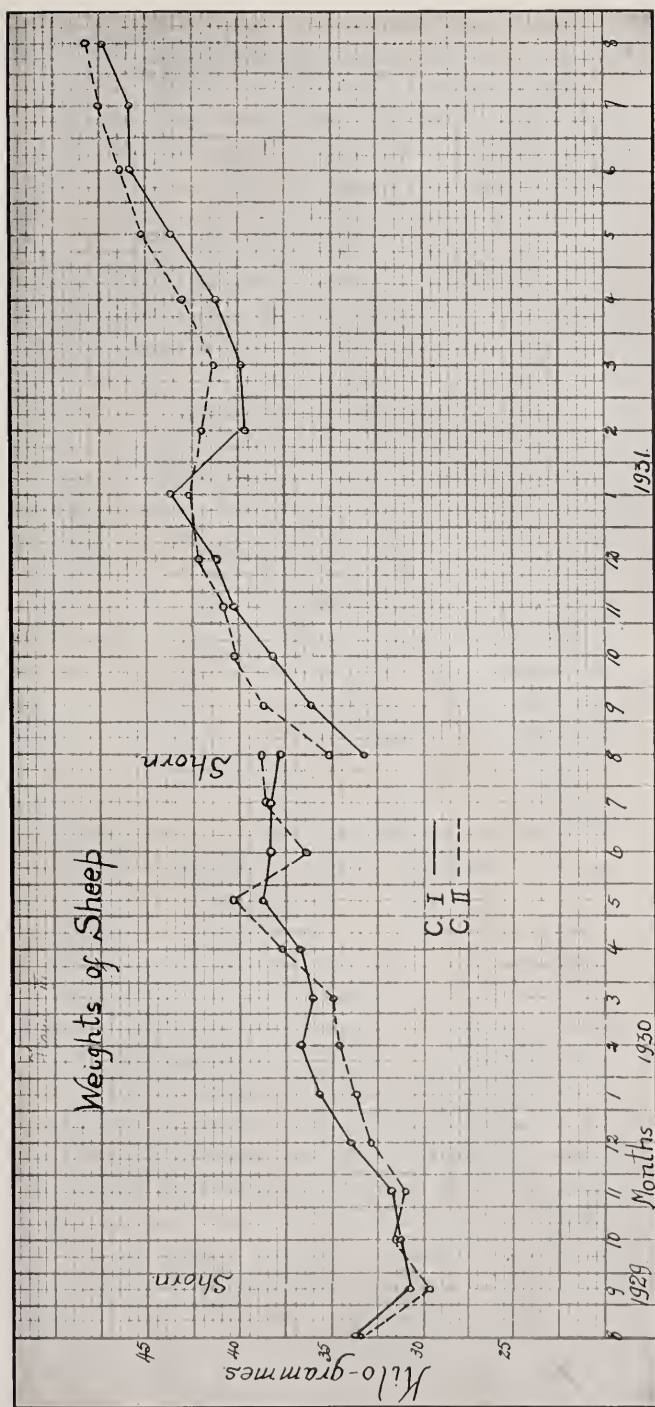


FIG. IV.

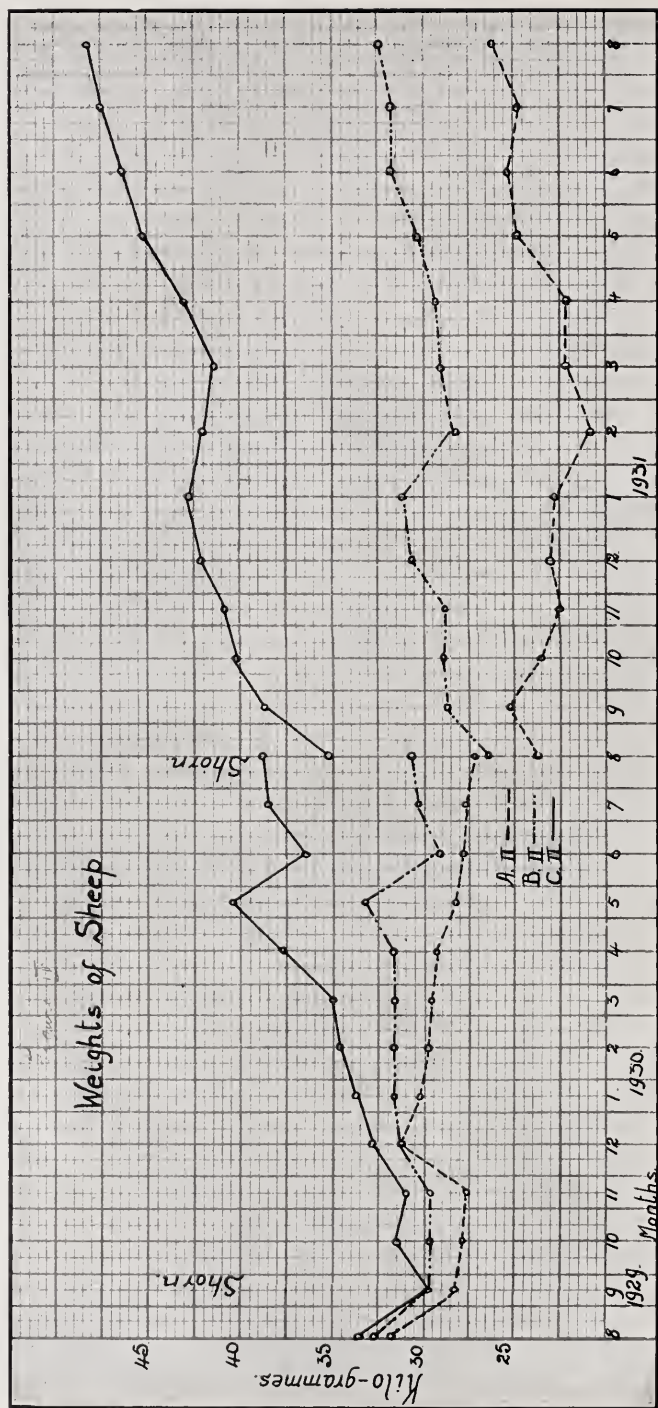


Figure IV hardly falls within the scope of this article. It is merely presented to note the differences in weights of the three subgroups getting the iodine supplement at different levels of phosphorus intake. This difference is perhaps better brought out in Figure V, where each dot represents the average monthly weight of an individual animal for the whole period of the experiment. Apparently the potassium iodide does not mask the effect of the phosphorus content of the ration.

(2) *Food Consumption.*—The graphs of the food consumption of the respective subgroups are given in the following figures:—

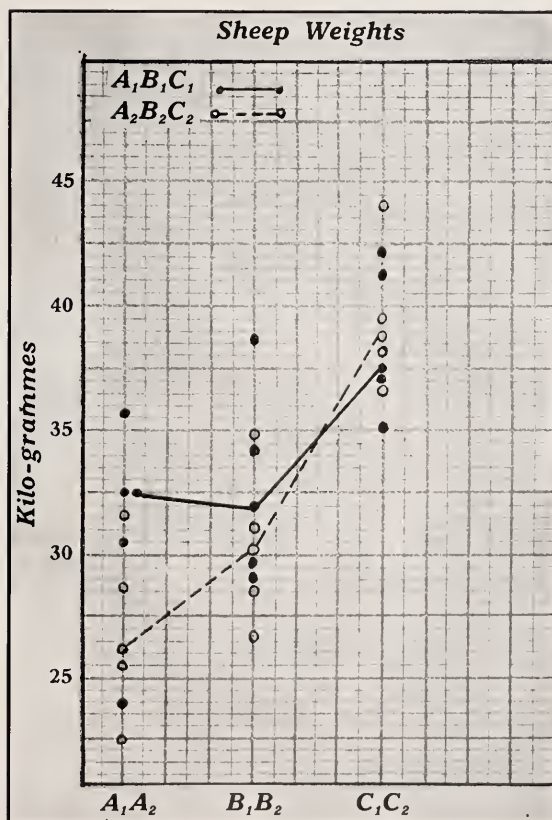


FIG. V.

FIG. VI.

Food Consumption.

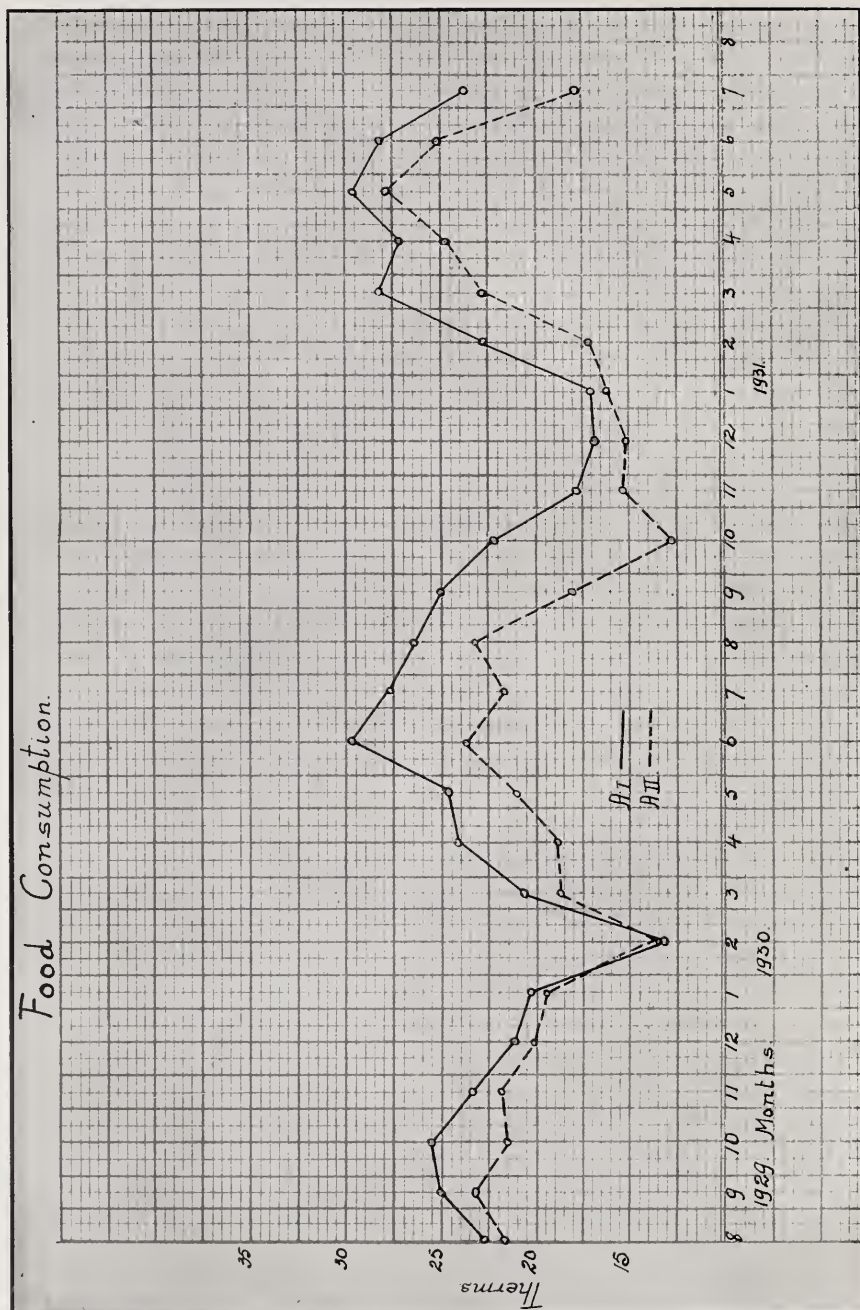


FIG. VII.

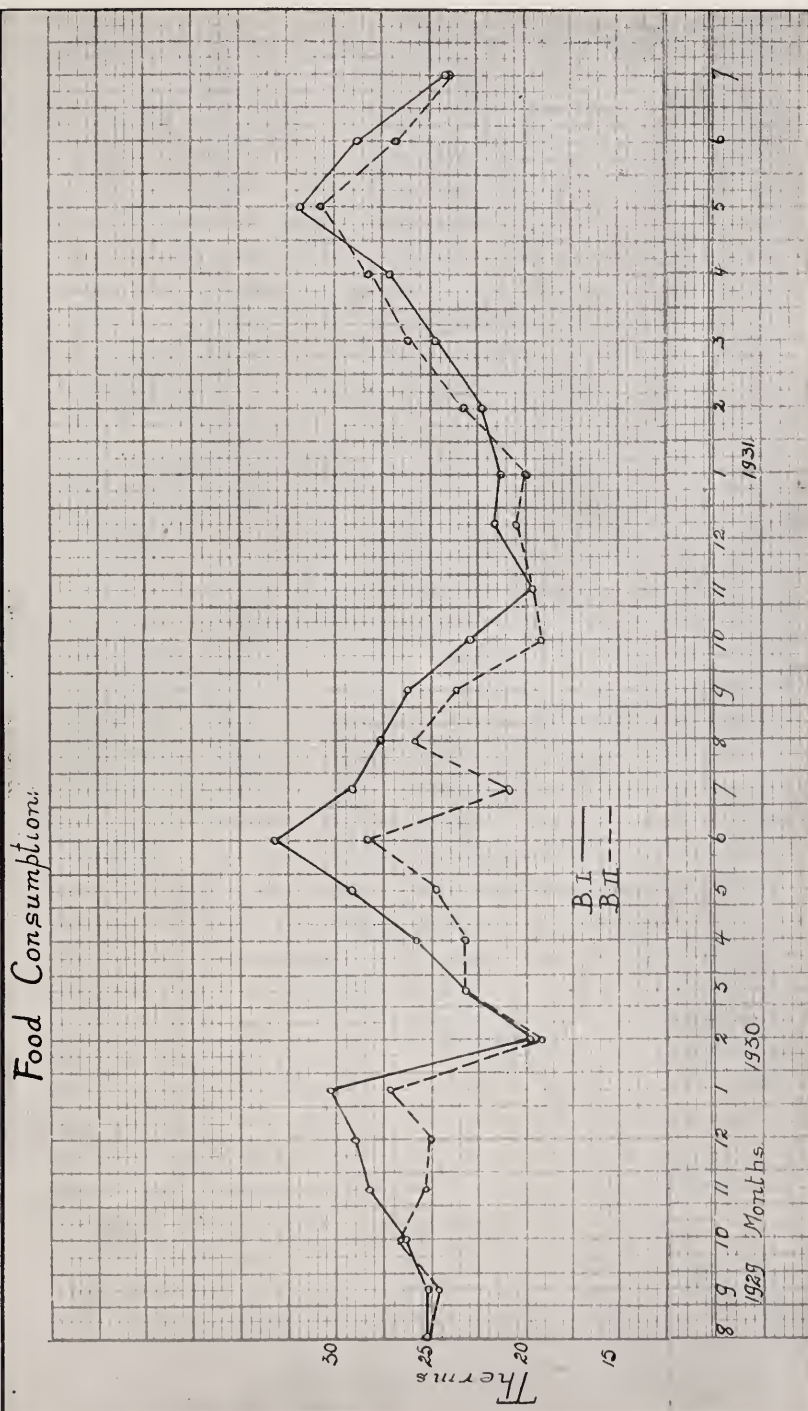


FIG. VIII

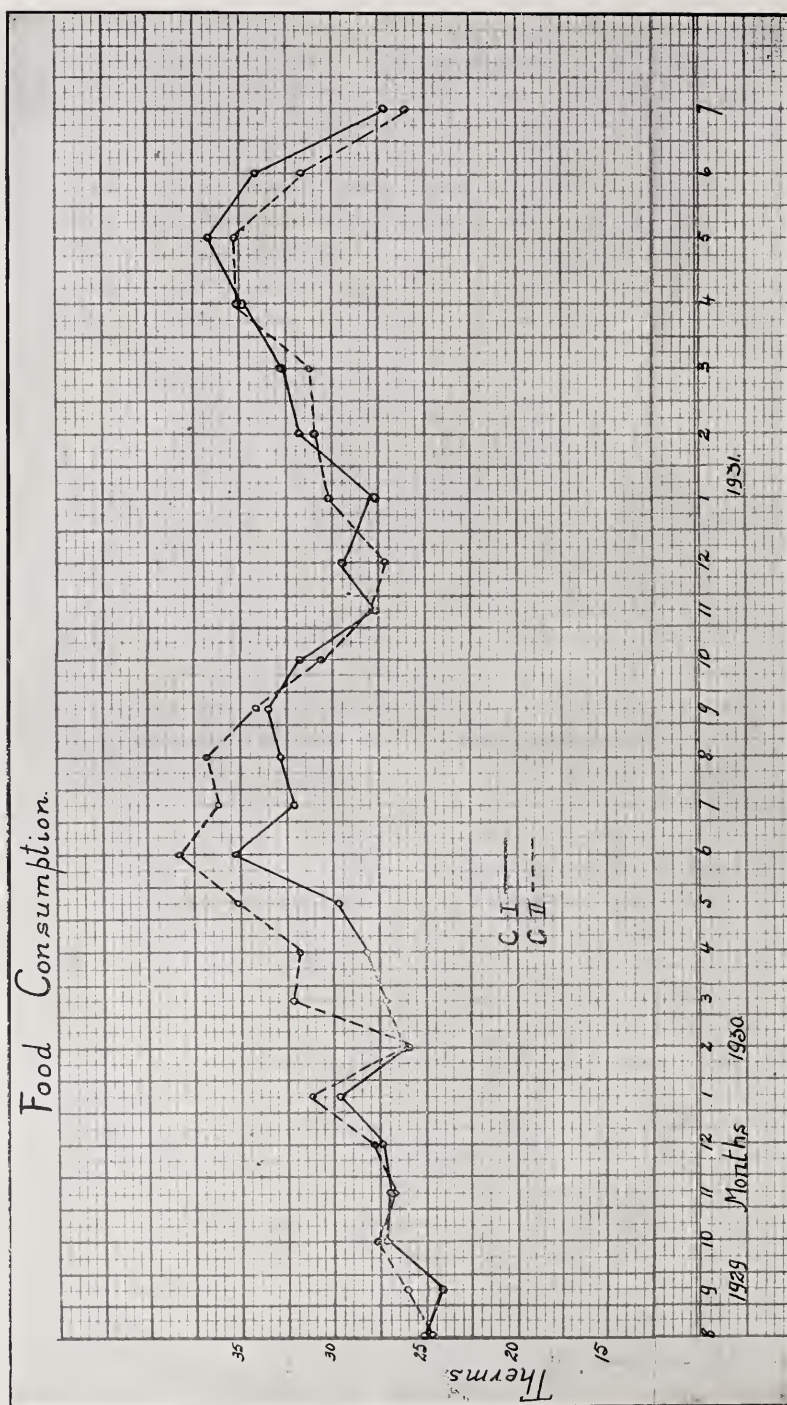
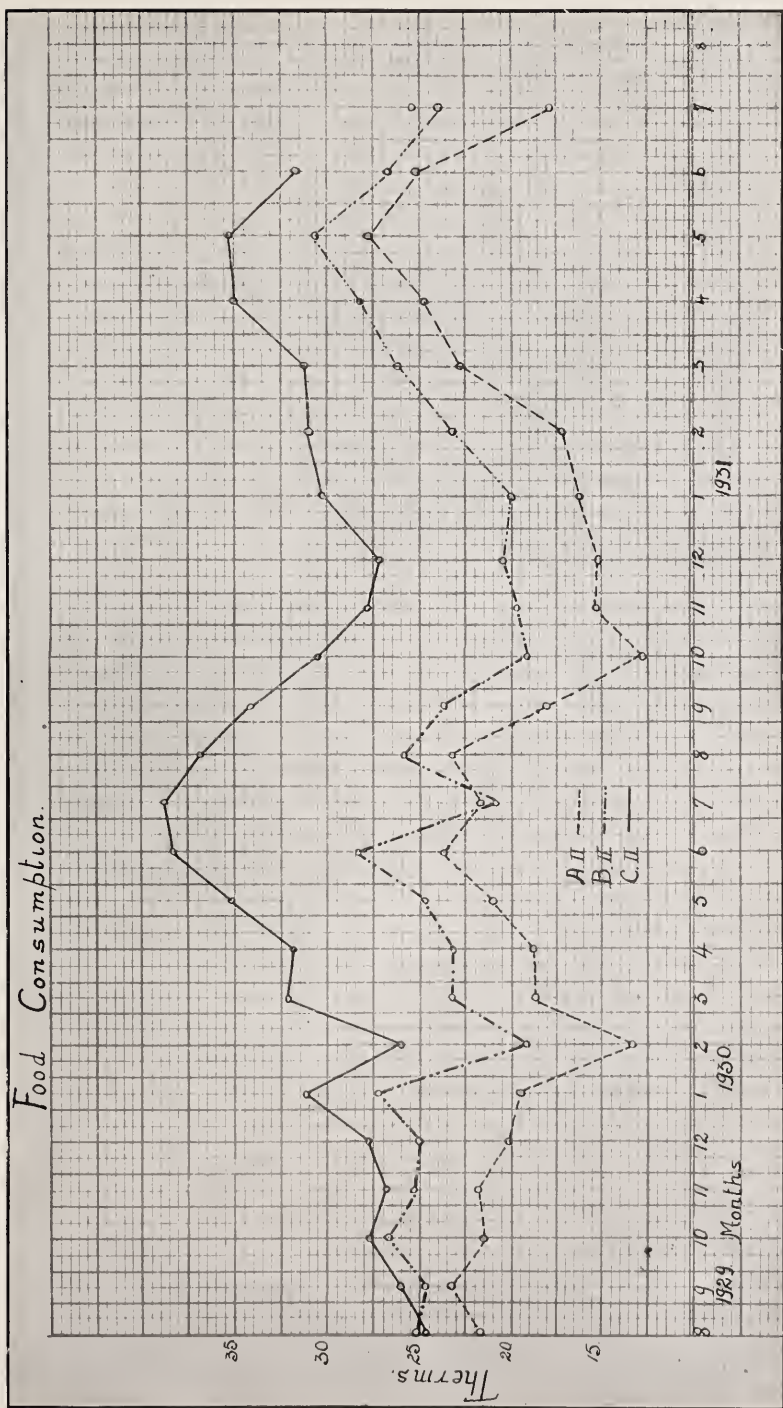


FIG. IX.



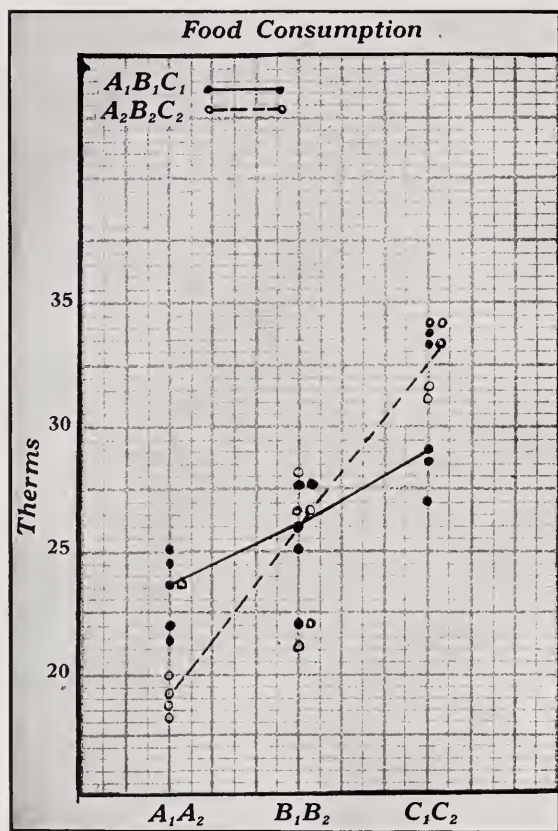


FIG. X.

(2) In figure VI a decided difference in the food consumption of the two subgroups is indicated, the lighter subgroup A_2 eating less throughout than its control A_1 . The probable significance of this difference of appetite will be left until reproduction is discussed. The amounts of food consumed by B_1 and B_2 do not differ significantly; if anything, subgroup B_2 ate slightly less. The same remarks apply to Figure VIII, where it appears that subgroup C_2 consumed on an average more food than subgroup C_1 . If the curves be considered in conjunction with the weight curves it is noticed that in a general way there is a marked agreement between these two factors. A_1 and A_2 show a decided difference in weight and this also applies to the amount of food consumed, A_1 the lighter group, consuming decidedly less food. The weights of B_1 and B_2 are less easily differentiated and the average quantities of food consumed show little difference. C_2 is slightly heavier than C_1 and consumed more food on the whole. The difference, however, is not big enough to warrant serious attention. The first drop in the food consumption of all six subgroups in February, 1930 was due to scab and dipping resulting in several deaths as stated earlier. The decided drop towards November, 1930, and again in July, 1931, cannot be accounted for. The weights do not show corresponding fluctuations. Figure IX represents the relative amounts of food consumed by the groups A_2 , B_2 and C_2 but the difference can, of course, not be said to be associated in any way with the iodine supplement. It is interesting from the point of view of the respective phosphorus intakes of the three subgroups. Figure X presents the same remarkable difference in a slightly different way and gives a comparison with the subgroups A_1 , B_1 and C_1 . There is no doubt that progressively less food was consumed as the phosphorus intake decreased from groups C to B to A. Furthermore, the subgroups receiving the iodine supplement responded peculiarly; C_2 actually ate slightly better than C_1 . In B_1 and B_2 the difference between the batch receiving iodine and its control is less significant and in A_2 the bad effect on food consumption is greatly intensified.

(3) *Inorganic phosphorus in the blood.*—Curves of the inorganic phosphorus in the blood of the animals are given in figures XI to XV.

FIG. XI

Inorganic Phosphorus in Hgm. per 100^{cc} Blood.

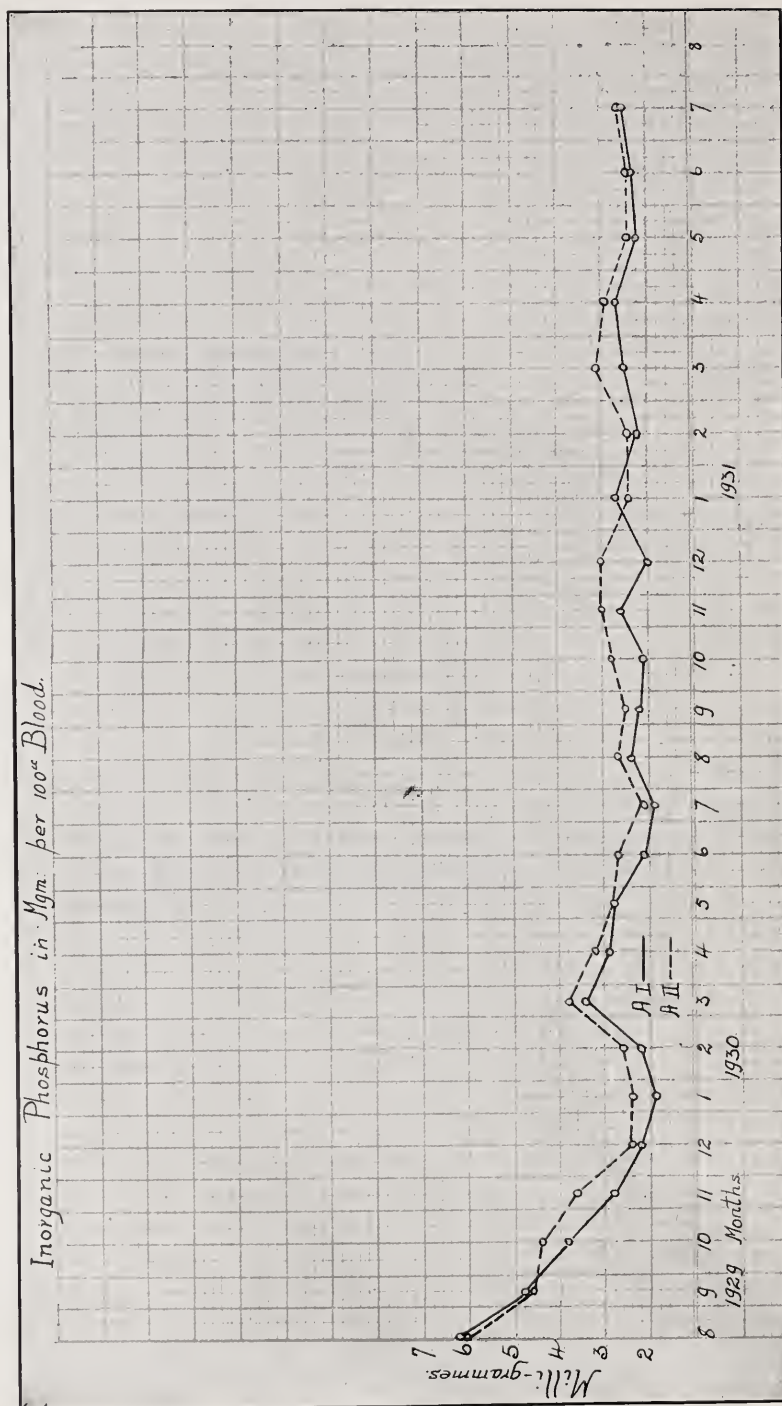


FIG. XII.

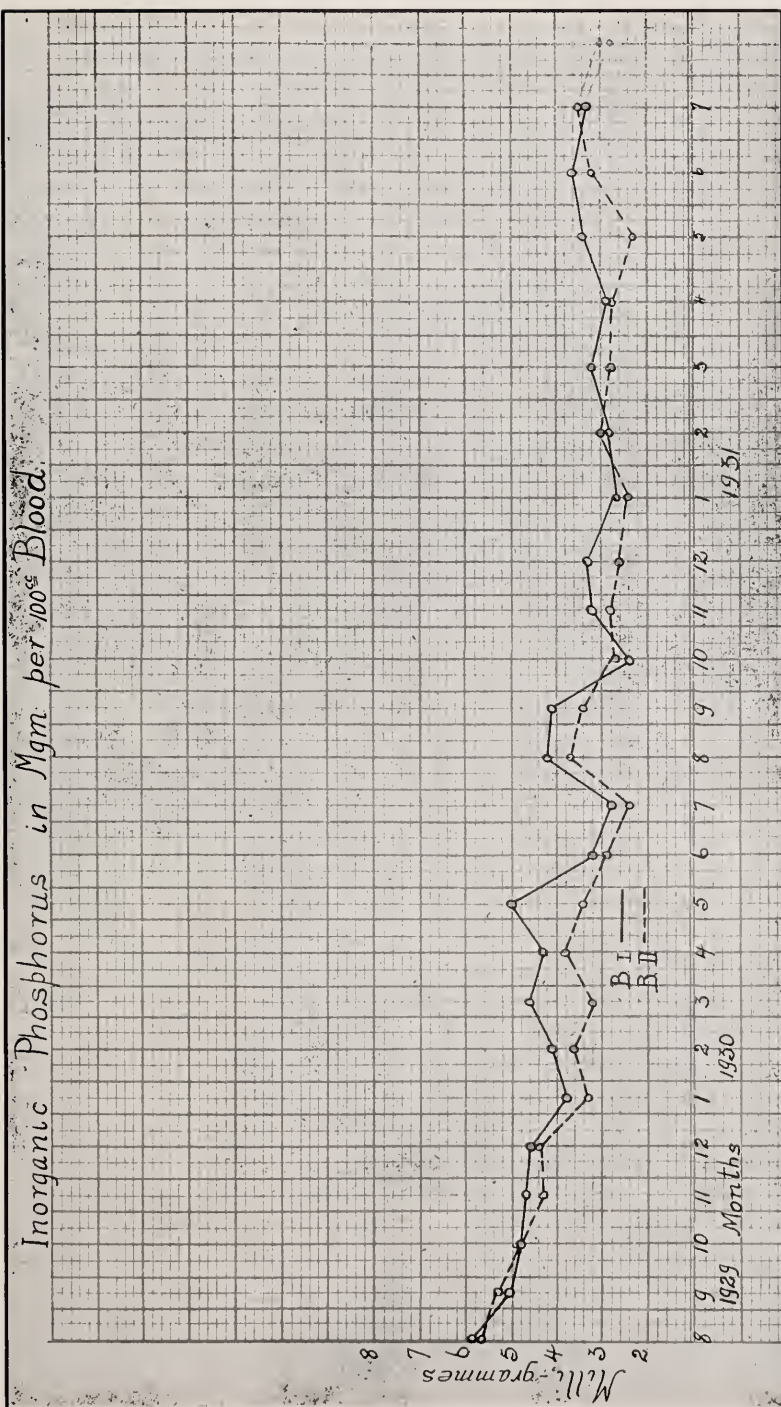


FIG. XIII.

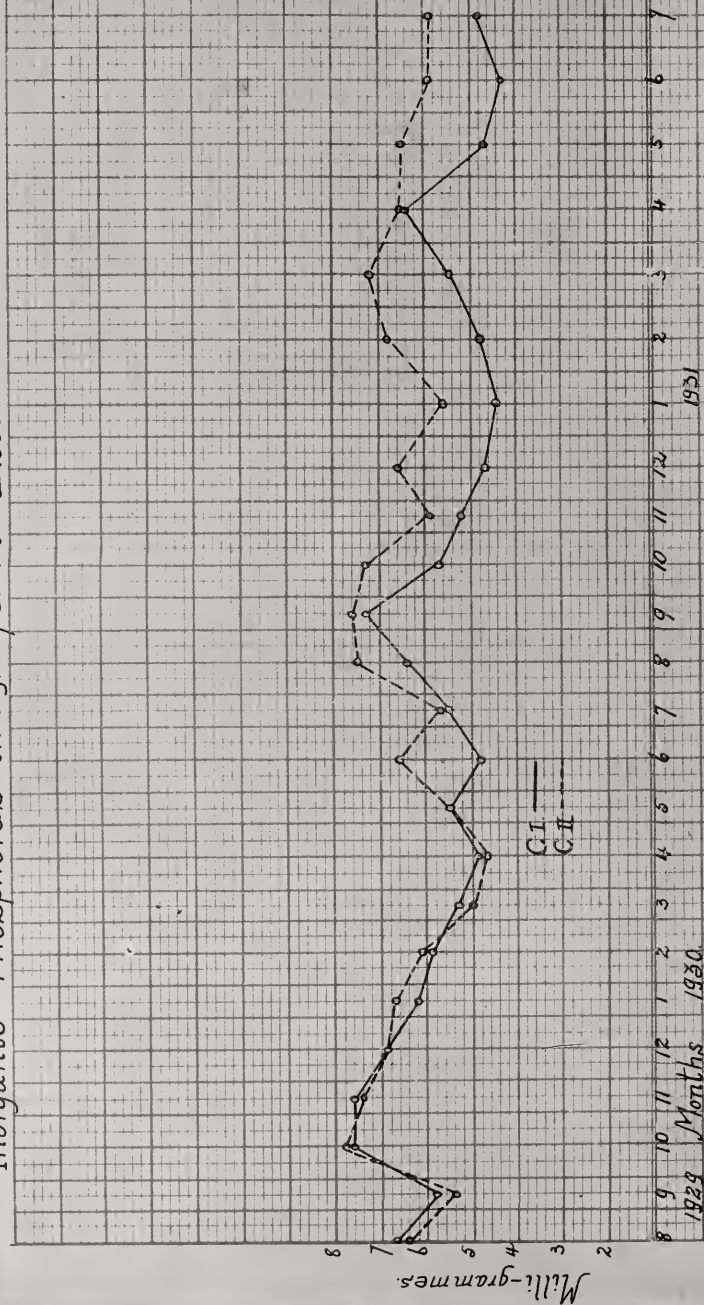
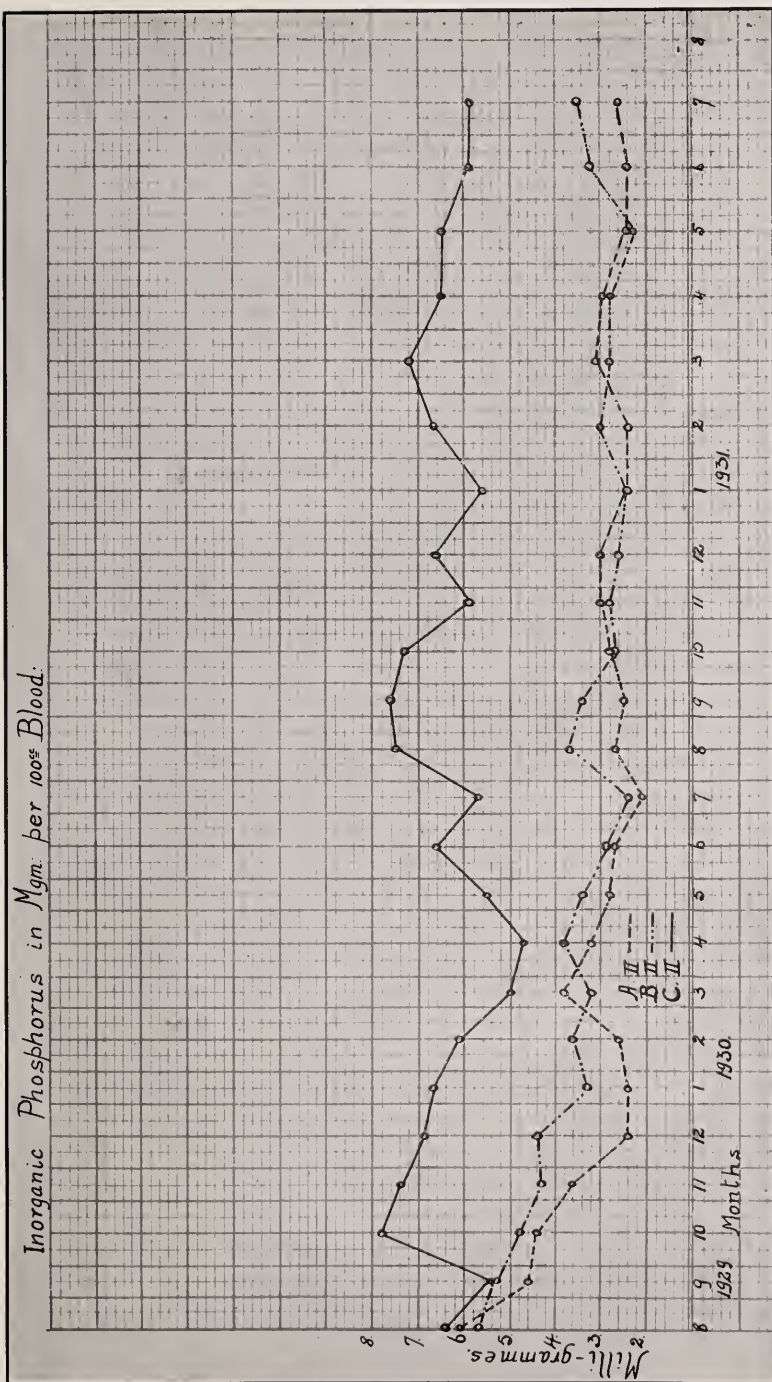
Inorganic Phosphorus in Mgm. per 100^{cc} Blood.

FIG. XIV.

Inorganic Phosphorus in Mgm. per 100^{cc} Blood.

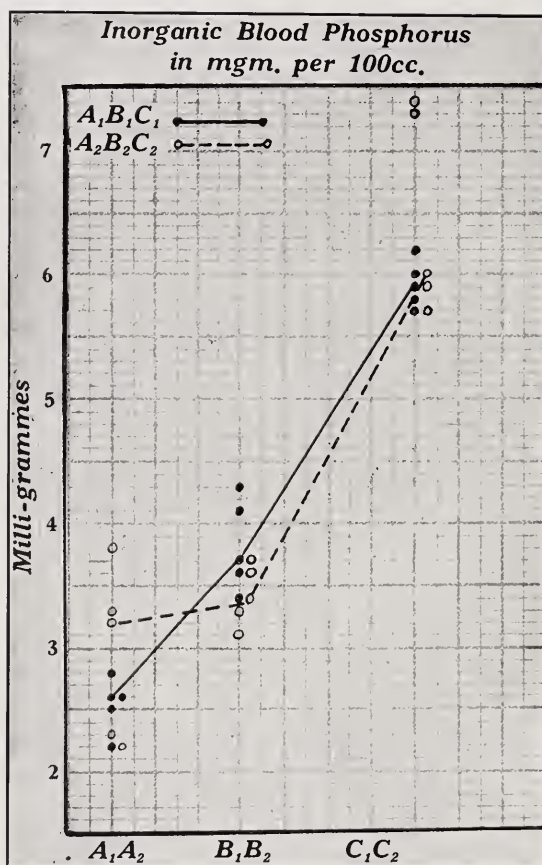


FIG. XV.

There is hardly any difference worth mentioning between the inorganic phosphorus of the groups receiving the iodine supplement and their respective controls. Subgroup A_2 shows a figure which is slightly higher than that obtained for A_1 . C_2 shows the same difference rather better during the last year of the experiment. If the differences were significant they would suggest better assimilation of phosphorus in the subgroups getting the iodide supplement. That does not appear to be true for group A_2 , judging from its weight increase, compared with that of A_1 . The difference between C_1 and C_2 will be discussed in connection with the data presented in Table XV.

TABLE 1.—LAMMING CHART FOR 1930 AND 1931.

1930.							1931.				
D.O.B. Nos.	Groups	Gestation Period, Days.	Birth Weight, lb.	Sex.	Final Weight, lb.	Remarks.	Gestation Period, Days.	Birth Weight, lb.	Sex.	Final Weight, lb.	Remarks.
26153.....	A ₁	—	—	—	—	Ewe not served.	153	4·7	—	7·6	Normal lamb.
23965.....	A ₁	—	—	—	—	No lamb.	151	6·3	—	11·6	Normal lamb.
23149.....	A ₁	—	—	—	—	Ewe not served.	150	5·3	—	9·9	Normal lamb.
24007.....	A ₁	—	—	—	—	Ewe not served.	153	5·3	—	—	Lamb died day of birth.
23968.....	A ₁	—	—	—	—	No lamb.	151	—	—	—	Lamb born dead.
Averages...		—	—	—	—		151·6	5·4	—	9·7	
23969.....	A ₂	150	6·2	♂	10·3	Normal lamb.	180	—	—	—	Putrified foetus removed.
26143.....	A ₂	—	—	—	—	Ewe not served.	95	—	—	—	No lamb.
26163.....	A ₂	152	5·9	♂	—	Ewe not served.	92	—	—	—	Twins aborted.
23972.....	A ₂	—	—	—	—	Lamb died aged 1 day.	115	—	—	—	Abortion.
23973.....	A ₂	—	—	—	—	No lamb.	—	—	—	—	Putrified foetus removed.
Averages...		151	6·05	—	—		162·5	—	—	—	
23974.....	B ₁	150	5·8	♀	11·1	Normal lamb.	—	—	—	—	No lamb.
23975.....	B ₁	152	5·4	♀	10·0	Normal lamb.	152	8·7	♂	15·5	Normal lamb.
26154.....	B ₁	—	—	—	—	Ewe not served.	—	—	—	—	No lamb.
26152.....	B ₁	—	—	—	—	Ewe not served.	150	4·9	♀	7·6	Normal lamb.
23978.....	B ₁	—	—	—	—	No lamb.	—	—	—	—	No lamb.
Averages...		151	5·6	—	10·5		151	6·8	—	11·5	

TABLE 1.—LAMMING CHART FOR 1930 AND 1931.—(continued.)

D.O.B. Nos.	1930.					1931.					
	Groups	Gesta- tion Period, Days.	Birth Weight, lb.	Sex.	Final Weight, lb.	Remarks.	Gesta- tion Period, Days.	Birth Weight, lb.	Sex.	Final Weight, lb.	Remarks.
24005.....	B ₂	—	—	—	—	No lamb.	141	—	—	—	Ewe aborted.
23980.....	B ₂	—	—	—	—	No lamb.	—	—	—	—	No lamb.
23981.....	B ₂	—	5.3	—	11.2	Normal lamb.	124	—	—	—	Ewe aborted.
23982.....	B ₂	149	6.8	♂	13.7	Normal lamb.	145	5.0	♂	—	Lamb died aged 1 day.
23983.....	B ₂	150	5.6	♂	9.2	Normal lamb.	149	4.6	♂	—	Lamb died aged 1 day.
Averages...		149.5	5.9	—	11.4		139.7	4.8	—	—	
26159.....	C ₁	—	—	—	—	Ewe not served.	143	5.8	♂	12.9	Normal lamb.
23985.....	C ₁	150	9.0	♂	14.9	Normal lamb.	152	6.4	♀	—	Lamb died aged 14 days
23986.....	C ₁	148	6.2	♂	14.2	Normal lamb.	148	5.7	♀	13.1	Normal lamb.
24008.....	C ₁	—	—	—	—	Ewe not served.	150	6.4	♀	6.7	Lamb abnormal, never able to walk.
23988.....	C ₁	—	—	—	—	No lamb.	159	8.0	♀	—	Lamb died aged 1 day.
Averages...		149	7.6	—	14.5		150.4	6.5	—	10.9	
23989.....	C ₂	—	4.5	—	10.7	Normal lamb.	147	5.2	♀	—	Lamb died aged 2 days.
23990.....	C ₂	—	6.7	♀	10.0	Normal lamb.	108	—	♀	—	Ewe aborted.
23991.....	C ₂	150	8.0	♂	14.8	Normal lamb.	137	3.6	♀	—	Lamb died aged 1 day.
23992.....	C ₂	151	6.8	♂	14.6	Normal lamb.	—	—	—	—	No lamb.
23993.....	C ₂	—	—	—	—	No lamb.	—	—	—	—	Ewe died heartwater 1/2/31; mummified foetus found in uterus.
Averages...		150.5	6.5	—	12.5		130.7	4.4	—	—	

Figures XIV and XV are given to show the difference in inorganic phosphorus for the separate groups. Figure XV shows a comparison with the controls and verifies, for the averages of the individual sheep, the statement made that the inorganic phosphorus of subgroup A₂ is slightly higher than that of A₁, and of C₂ than that of C₁. It may be pointed out that two sheep in C₂ show remarkably high phosphorus for the whole period as shown in Table XV. This explains the marked difference in the two groups in Table XIII and at the same time makes the difference less significant.

(4) *Reproduction*.—The comparative lambing chart for 1930 and 1931 is reproduced in Table 1.

A careful study of the lambing chart reveals a number of interesting points in spite of the many gaps which it contains. Although the lambing season of 1930 taken as a whole, was a failure with regard to the number of lambs produced, there is nothing to suggest abnormal conditions. Lambing took place about 8 months after the commencement of the experiment and 3 months after the outbreak of scab, which necessitated the substitution of nine fresh ewes, which, owing to the lateness of the season, were not put to the ram. The lambs were removed permanently from the ewes 21 days after birth. It must be kept in mind that the irregularity of lambing must have affected the groups differently and that, later response to experimental conditions might be affected. A summary of the results of the lambing season 1930 is given in Table 2.

TABLE 2.

Groups.	A ₁ .	A ₂ .	B ₁ .	B ₂ .	C ₁ .	C ₂ .
Possible No. of lambs.....	2	3	3	5	3	5
Lambs produced.....	0	2	2	3	2	4
Gestation period (days).....	0	151	151	149·5	149	150·5

It seems as if there was a tendency for the subgroups receiving the iodine supplement to produce more lambs than the other subgroups, but there is no indication that the gestation period was affected.

Proceeding to the lambing season in 1931, that is 8 months afterwards and 18 months from the beginning of the experiment, it is at once noticeable that abnormalities occurred. Outstanding points and comparisons are summarised in Table 3.

TABLE 3.

Groups.	A ₁ .	A ₂ .	B ₁ .	B ₂ .	C ₁ .	C ₂ .
Possible No. of lambs.....	5	5	5	5	5	5
No. of pregnancies.....	5	4	2	4	5	4
Gestation period.....days	151·6	102·5	151	139·7	150·4	130·7
No. of live lambs produced....	4	0	2	2	5	2
No. of lambs 1 day after birth.	3	0	2	2	5	2
No. of lambs 3 days after birth	3	0	2	0	4	0
No. of lambs 21 days after birth	3	0	2	0	3	0
Weights of lambs at birth...lb.	5·4	—	6·8	4·8	6·5	4·4
Weights of lambs 21 days old...lb.	9·7	—	11·5	—	13·0	—

Table 3 and the lambing chart for 1931 cannot be passed without comment. Of 12 pregnant ewes in the subgroups receiving the iodide supplement, only 4 lambs were born, of which one, at least—lamb of ewe No. 23991—was premature. In any case, none of these lambs lived longer than two days, and their weights at birth suggest weakness, if anything. Of the control groups 12 pregnancies produced 11 lambs, of which 8 lived longer than 21 days, when lactation was terminated in order to subject the groups to the same treatment as much as possible. The weights of the lambs 21 days after birth provide a measure of the milk yield of the ewes. The lamb of ewe No. 24008 in C_1 lived for the whole period, but could never stand—the muscles of the forelegs were apparently undeveloped. Its weight is, therefore, excluded from the averages of final weights. In a general way the final weights indicate that milk yield increases with increase of phosphorus in the ration, i.e. from subgroups A_1 to B_1 to C_1 on a phosphorus sufficient ration. Such increase has been shown time and again for lactating cows. The difference in birth weights of the lambs of A_1 , B_1 and C_1 may or may not be significant; the data appears to be inadequate for any conclusions when the individual variations are considered. For the same reason any inference based on the final weights could not be made seriously. Apparently abortion in subgroups A_2 on an exceedingly low phosphorus diet with an iodine supplement was brought about sooner than in the other groups. The foetuses were very small and partly decayed, whereas in B_2 and C_2 the condition resembled normal reproduction more closely.

It is remarkable, however, that a very high percentage of ewes aborted or had to have the foetuses removed in the groups receiving the iodine supplement, i.e. 8 of the 12 or 66 per cent., and of the remaining ewes it is extremely doubtful whether there was a single one that had given birth to a normal lamb. Of the control groups one ewe aborted a full-time foetus, and two day-old lambs died, one of which—that of ewe No. 23988 in Group C_1 —was long overdue, very big and gave great difficulty at birth. As a matter of fact, only expert assistance kept the lamb alive during the procedure. The fact remains that there can be no doubt that some factor influenced reproduction adversely in A_2 , B_2 and C_2 , a factor which was by no means present to anything like the same extent in the subgroups A_1 , B_1 and C_1 . Whether the detrimental effect was entirely due to the iodine administered is difficult to state positively, but that the iodide seemed to aggravate the state of affairs is almost a certainty, and apparently much more so in the group receiving a minimum of phosphorus in its ration.

The number of pregnancies in all the subgroups except B_1 was fairly satisfactory. Of the three ewes in B_1 that did not lamb in 1931, two had not produced lambs in 1930. This also applies to ewe No. 26143 in A_2 and to No. 23980 in B_2 . Although these omissions are comparatively few, and do not affect the reproductive side of the experiment seriously, they must have caused a certain amount of irregularity in the groups, and it becomes essential therefore to reconsider some of the figures of weight increase and food consumption in the light of this knowledge.

Figure V shows that taken as a group A_1 withstood the phosphorus deficiency much better than A_2 . The average weight of these two respective subgroups given in Figure 1 leave no doubt which is the lighter group. Still, it must be remembered that A_2 gave birth to two lambs in 1929, whereas none were born in A_1 . There is, of course, the fact that in 1931 A_1 reared three lambs for 21 days, whereas A_2 had none to provide milk for. It seems hardly likely that the physiological disturbance of abortion could have been responsible for the comparatively greater setback that A_2 experienced. Table I does not support such a view, for the inferiority of A_2 is shown to have existed almost from the start. Figures IV and X confirm the observations for food consumption, as they ate better than A_2 throughout. There seems to have been an extra factor absent in A_1 which caused the setback in A_2 . A comparison of B_1 and B_2 is interesting. B_1 had four pregnancies for the two lambing seasons and suckled all four lambs for 21 days. B_2 had seven pregnancies, while three lambs remained with the ewes for the full 21-day period. On the face of these facts, not much difference in weight increase between these two subgroups would be anticipated. Figures II and V confirm these findings, which are borne out in Figures VII and X, giving the respective curves for food consumption. The differences in weight and food consumption between these two groups may therefore be called incidental and cannot account for the difference in reproduction between these two groups.

Subgroups C_1 and C_2 are also clearly distinguishable when the lambing chart in Table I is studied. A comparison of C_1 with its eight pregnancies for the two seasons and subsequent lactation with C_2 , where nine ewes were due to lamb but no lactations to follow, suggests that C_2 was slightly favoured by this state of affairs. A glance at Figures III, V and VIII confirms such a view.

In conclusion it may therefore be pointed out again that the greatest difference between the control groups and those receiving the iodide supplement lies with reproduction, where no ground can be found for acclaiming the beneficial effects of iodide administration. As a matter of fact, although reproduction in the control groups was by no means ideal, that in the groups receiving the iodide was poor enough to suggest very strongly that the iodide had harmful effects at the second lambing season. The quantity of potassium iodide must not be lost sight of when considering the results. There is always the possibility, of course, that a smaller dose might not have produced harmful effects. In another experiment now in progress with four groups of ewes varying quantities of potassium iodide are given in order to investigate this matter further.

SUMMARY.

1. 0.02 gm. potassium iodide was given as a daily supplement to three groups of Merino ewes at three levels of phosphorus intake. Each group had its respective control.

2. The phosphorus content of the ration of the three groups was equivalent to (a) one as low in phosphorus as it could be made with the basal ration in question, (b) equivalent to the intake of sheep on grass in phosphorus-deficient areas in South Africa, (c) equivalent to the intake of sheep on good quality natural grass.

3. The experiment was conducted for 24 months and data are presented giving the weight increase, food consumption, inorganic phosphorus in the blood and reproduction for the entire period.

4. Except in the groups on the lowest phosphorus level there is no significant difference between the groups receiving iodine and the controls, if reproduction be excluded. What difference there is could be anticipated from a comparative study of the chart giving reproduction for 1930 and 1931.

5. The group on the lowest phosphorus level receiving the iodide supplement is decidedly lighter and consumed less food than its control. Unless the physiological disturbance of abortion is responsible for the additional setback it seems that the iodide supplement intensified the detrimental effects of phosphorus deficiency or brought about additional detrimental results.

6. The inorganic phosphorus content of the blood was lowest for the group on the lowest level of phosphorus intake and increased with increase of phosphorus content of the ration. The supplement of KI apparently left the phosphorus content of the blood unaffected except in case of two sheep in Group C₂.

7. The lambing season in 1930 showed no significant differences between the groups receiving iodine and the controls except, may be, that more ewes lambed in the former groups.

8. In 1931 remarkable differences were observed between the controls and the groups receiving KI. In the latter groups

- (a) 4 lambs were produced as against 11 in the controls,
- (b) 8 ewes aborted or had the foetuses removed as against 1 full-time abortion in the controls.
- (c) Not a single lamb was alive on the 3rd day as against 9 in the control groups.
- (d) The number of pregnancies does not seem to have been affected.

9. It is concluded that a daily dose of .02 gm. KI had a detrimental effect on reproduction in 1931, i.e. 16 months after the commencement of the experiment.

REFERENCES.

- CARROLL, E. W., MITCHELL, N. H., and HUNT, G. E. (1930). Potassium iodide as a mineral supplement in paired feeding experiments with growing swine. *Jnl. Agric. Research*, Vol. 41, No. 1, pp. 65-77.
- DU TOIT, P. J., MALAN, A. I., and GROENEWALD, J. W. (1931). 17th *Rept. Dir. Vety. Serv. and Anim. Indust., Union of S.A.*, pp. 454-472.
- DU TOIT, P. J., MALAN, A. I., and ROSSOUW, S. D. (1930). 16th *Rept. Dir. Vety. Serv. and Anim. Indust., Union of S.A.*, pp. 313-326.
- EVVARD, J. M. (1928). Iodine deficiency symptoms and their significance in animal nutrition and pathology. Lecture delivered at 12th Annual Session, The Association for the Study of Internal Secretion, Minneapolis, June, 1928.
- GOLF, A., and BIRNBACH, G. (1927). Ein Fütterungsversuch mit Jodkali auf Zibben—und Hammellämmern. *Zietsch. für Schafzucht*, Vol. 8, pp. 1-5.
- ORR, J. B., CRICHTON, A., and MIDDLETON, W. (1929). A note on the toleration of farm animals for high doses of iodine. *Vet. Record*, Vol. 9, No. 4, 1929.
- ORR, J. B., and LEITCH, I. (1929). Iodine in Nutrition—A review of existing information. Pub. by His Majesty's Stationery Office.
- TINLIE (1922). Quoted by Orr and Leitch.

Studies in Mineral Metabolism XXI.

A Comparison of Phosphatic Supplements for The Prevention of Aphosphorosis.

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THE importance of feeding supplements containing phosphorus to cattle in phosphorus-deficient areas in the Union has been stressed on many occasions by the Division of Veterinary Services [Theiler, Green and Du Toit (1924) (1928), Du Toit and Green (1930), Du Toit and Bisschop (1929), Bekker (1932)]. In fact it has been shown that, whereas the cattle industry was threatened with extinction in large areas in Bechuanaland not more than a dozen years ago, it is the mainstay in those areas now, thanks almost exclusively to the feeding of bonemeal at the rate of a few ounces per day.

Next in importance came the method of giving phosphatic supplements and their nature; and it was with the object of obtaining more information on this phase of the problem that the investigation reported on in the present paper was undertaken.

Various products containing phosphorus have been on the market, but as most of the results showing the advantages of phosphorus feeding have been obtained with bonemeal, the price of this product has increased and its use extended to the exclusion of other materials that are apparently as efficacious and less expensive. Then too, deposits of rock phosphate have been discovered in the Union, but rock phosphate has generally been reported to be injurious to stock (Du Toit *et al.*, 1932), in spite of which it is being used by some stockmen in this country. Furthermore, the question of feeding phosphates in a water soluble form and therefore in the drinking water of cattle has frequently been raised, although its practicability has never been investigated; an experiment on the relative value of phosphatic supplements would, of course, include both rock phosphate and a water soluble compound. Such an investigation was, therefore, begun in October, 1929, on the lines detailed below.

EXPERIMENTAL WORK.

Fifty-eight young oxen and heifers were selected and divided into nine groups. These animals were about nine months old and had just been weaned. Each group except two which were kept as controls and for the administration of the water soluble phosphate respectively, was dosed with a phosphatic supplement daily except Sundays as outlined hereunder. The animals were kept only on pasture on the Government farm Armoedsvlakte in the well-known phosphorus-deficient area of Bechuanaland. Each group consisted of tollies and two heifers and were treated as follows:—

- Group 1.—7 animals received $\frac{3}{4}$ oz. precipitated calcium phosphate daily, i.e. 8.0 gm. P_2O_5 .
- Group 2.—7 animals received 2 oz. bonemeal, i.e. 12.5 gm. P_2O_5 .
- Group 3.—7 animals received 1.5 oz. degelatinized boneflour, i.e. 12.5 gm. P_2O_5 .
- Group 4.—7 animals received 3 oz. basic slag daily, i.e. 14.3 gm. P_2O_5 .
- Group 5.—6 animals received 2 oz. superphosphate daily, i.e. 9.7 gm. P_2O_5 .
- Group 6.—8 animals received 2.0 oz. powdered rock phosphate daily, i.e. 16.5 gm. P_2O_5 .
- Group 7.—8 animals received 1.5 oz. disodium phosphate daily, i.e. 8.6 gm. P_2O_5 .
- Group 8.—7 animals received 1.5 oz. disodium phosphate via drinking water daily, i.e. 8.6 gm. P_2O_5 .
- Group 9.—8 animals received no supplement.

All these animals except those in group 8 were treated exactly alike. For instance, they grazed in the same camps, were collected daily for dosing at the same time, and were handled in the crushes and for pica-testing to the same extent. Even the controls were made to walk through the crushes, although they were not dosed, of course.

Group 8 was moved to a different paddock where facilities for drinking water could be so arranged that the animals had free access to it. Briefly the arrangement was as follows: A small circular concrete tank of known capacity was built and connected with the main water supply. This tank was filled and disodium phosphate added at the rate of 1.5 oz. per average amount of water taken daily by each animal. One tank full of water lasted about a fortnight and for the next period the water consumption of the previous fortnight was used to calculate the average daily water consumption per beast for making up the phosphate solution. The drinking water in question contained about 180 mgm. calcium per litre and about 40 mgm. of magnesium. Hence the precipitation of phosphate had to be obviated by the addition of a small quantity of sulphuric acid. It was found after some experimentation that 1 c.c. concentrated sulphuric acid per gallon of water prevented the formation of a precipitate, but was not enough to impart an acid reaction to the water.

The animals showed no disinclination whatsoever to drink the water containing the phosphate. It had a pleasant taste and not very unlike that of the water without the phosphate. However, the animals definitely preferred this water after the conclusion of the experiment when they took several days to get accustomed to the water from the main supply. During the course of the experiment the animals in group 8 were allowed free grazing in the camp in question, were left undisturbed day and night and collected only once a fortnight for pica-testing and weighing. It is important to note that group 8 was spared the daily driving to crushes which, in the ordinary course of events must demand on the part of the animals a fair amount of energy that can hardly be spared in times of drought and scarcity of food. It must further be added that all the groups except 8 were allowed about 20 acres of grazing per head and that the animals in group 8 had about 30 acres each at their disposal. This factor will again be considered when the results are discussed. It must also be mentioned that a full account of the effects of disodium phosphate on the growth of the animals in group 8 is given by Bekker (1932) and reference must be made to his publication for a detailed study of that particular group, for the inclusion of which in this investigation Bekker was largely responsible. In this article general comparisons will be drawn from a study of the various groups primarily with a view to show (*a*) the relative values of the two methods of phosphate feeding, via the water supply and dosing, and (*b*) the practical application of the feeding of phosphatic products obtainable but not yet utilized.

The group receiving precipitated calcium phosphate is regarded as the standard group for comparison. The daily dose $\frac{2}{3}$ oz. has given good results in the past (Du Toit and Green 1930), and its effects are

only slightly inferior to those of 3 oz. of bonemeal. From the work of Du Toit and Green (1930) already quoted, there appears to be no doubt that the phosphorus of precipitated calcium phosphate is more available to the animal than that in bonemeal. As a matter of fact, working with a young ox in balance experiments Otto (1932) found that all the phosphorus in precipitated calcium phosphate was absorbed, whereas the proportion of that absorbed from bonemeal was considerably less. Working on these bases, therefore, it was decided that, if $\frac{2}{3}$ oz. of precipitated calcium phosphate or 8 gm. P_2O_5 was probably the daily requirement of an animal, 2 oz. of bonemeal might contain an equivalent quantity of available P_2O_5 . Also, Du Toit and Green were inclined to believe that the optimum dose of bonemeal for growth was less than 3 oz. per day.

Group 3 received as much P_2O_5 in the form of degelatinized bone-flour as group 2 did in the form of bonemeal. Group 4 with its supplement of basic slag is more or less on the same basis, i.e. like bonemeal only partly available. The supplements in groups 5, 7 and 8, viz., superphosphate, sodium phosphate, dry, and sodium phosphate solution respectively, are regarded as directly comparable with that of group 1, viz., precipitated calcium phosphate, which can hardly be regarded as being more available than the supplements in any of the three groups mentioned. Hence the daily P_2O_5 intake in these groups are about the same. In view of the belief of Theiler *et al.* (1927) that finely ground rock phosphate has a much lower availability than bonemeal, the dose of this material was increased to contain 16.5 gm. P_2O_5 , for even in doses as large as this the product in question would be considerably less expensive than most of the others. Another consideration for giving a comparatively large dose of ground rock phosphate was the fact that it contains about 3.5 per cent. of fluorine, which has been reported to be injurious to stock (Du Toit *et al.*, 1932). It therefore became necessary to ascertain if excessive doses of rock phosphate might not produce harmful effects.

The experiment may be divided into two phases:—

- (1) From October, 1929, until May, 1930, during which time the doses were those given on page 678.
- (2) May, 1930, until the conclusion of the investigation in January, 1932.

For the latter period some of the doses were made distinctly larger with the view to produce harmful effects if the materials used should be toxic when given in larger quantities. At the same time the daily doses of nearly all the remaining products were slightly increased to what was regarded as the desired quantities for optimum growth as judged by the pica or oestophagia tests, and by the weight increase of the period under review. In the case of the group receiving sodium phosphate—dry compound—the daily dose was increased to 2.5 oz. for the period May, 1930, to August, 1930, in an attempt to reduce osteophagia. After that the original dose of 1.5 oz. was again given.

An additional group was formed by selecting animals from the largest groups and a second brand of bonemeal given. The arrangement of the groups after May, 1930, was thus as follows:—

- Group 1.—Precipitated calcium phosphate increased to 1 oz. daily or 10·7 gm. P_2O_5 .
- Group 2.—Bonemeal increased to 3 oz. daily or 16·7 gm. P_2O_5 .
- Group 3.—Degelatinized boneflour increased to 2 oz. daily or 16·7 gm. P_2O_5 .
- Group 4.—Basic slag increased to 5 oz. daily or 23·9 gm. P_2O_5 .
- Group 5.—Superphosphate increased to 4 oz. daily or 19·4 gm. P_2O_5 .
- Group 6.—Ground rock phosphate increased to 4 oz. daily or 33·0 gm. P_2O_5 .
- Group 7.—Disodium phosphate increased to 2·5 oz. until August, 1930, i.e. 13·7 gm. P_2O_5 .
- Group 8.—Remained constant, i.e. 8·6 gm. P_2O_5 daily.
- Group 9.—Controls.
- Group 10.—Bonemeal B given 3 oz. daily or 16·7 gm. P_2O_5 .

RESULTS OF EXPERIMENT.

The curves representing the average group weights, the tables giving percentage craving or pica calculated on the basis explained by Du Toit and Green (1930) and the average inorganic phosphorus in the blood are given at the end of this publication. As the curves are too numerous for comparative plotting on a few sheets tables have been compiled which attempt to present a complete picture of the results obtained in all the groups for the whole period. A consideration of these tables together with a discussion of the separate groups will make group comparison easier and common features more obvious.

For the sake of convenience and to simplify making comparisons the experiment will be divided into periods and then taken as a whole:—

(1) October, 1929, to May, 1930: October to May is the time of the year that greatest growth takes place. As a rule rains have fallen and pasture is abundant. Incidentally during this period in 1929-1930 smaller doses of supplements were given than after May, 1930.

(2) May, 1930, to October, 1930: This period includes winter and early spring with poor grazing, which resulted, as is usually the case, in a general drop in the weight of the animals.

(3) October, 1930, to May, 1931, shows, like period (1), an increase in the weights of the animals.

(4) May, 1931, to October, 1931, is directly comparable with period (2) with its associated drop in weight.

(5) October, 1929, to January, 1932: The same considerations for the separate periods will be applied to the full time of the experiment.

TABLE 1.

AVERAGE WEIGHTS, IN POUNDS, OF GROUPS FOR PERIODS GIVEN.

Groups.	Oct., 1929.	May, 1930.	Oct., 1930.	May, 1931.	Oct., 1931.	Jan., 1932.	Gain, Oct.-May, 1929-30.	Gain, Oct.-May, 1930-31.	Gain, May-Oct., 1931.	Gain, Oct.-Jan., 1929-32.	% Gain, Oct.-Jan., 1929-32.
CaHPO ₄	360	565	557	846	833	997	205	289	-13	637	178.0
Bonemeal "A".....	362	622	618	917	893	1,057	260	299	-24	695	191.9
Deg. Bonedflour.....	354	564	576	857	843	998	210	281	-14	644	181.9
Basic slag.....	382	567	546	817	774	940	185	271	-43	558	146.1
Superphosphate.....	368	553	484	840	767	887	185	356	-73	519	141.0
Rock phosphate.....	375	486	415	—	—	—	111	—	—	—	—
Na ₂ HPO ₄ dosed.....	354	583	586	862	850	1,000	229	276	-12	646	182.5
Na ₂ HPO ₄	365	664	667	—	—	—	299	—	—	—	—
Controls.....	429	551	525	710	679	785	122	185	-31	356	82.9
Bonemeal "B".....	—	612	617	872	891	1,041	—	255	+19	—	—

TABLE 2.
PERCENTAGE PICA AND INORGANIC PHOSPHORUS (I.P. IN MGM. PER 100 C.C. BLOOD).

	October-May, 1929-30.		October-May, 1930-31.		May-October, 1930.		May-October, 1931.		October-January, 1929-32.	
	I.P.	Pica.	I.P.	Pica.	I.P.	Pica.	I.P.	Pica.	I.P.	Pica.
CaHPO ₄	4.6	77.5	49.5	5.6	5.8	59.1	29.6	5.7	5.4	55.5
Bonemcal "A".....	4.9	41.6	26.1	5.9	5.8	32.9	14.3	5.6	5.6	30.1
Deg. Bonemcal.....	5.2	39.1	31.1	5.5	5.8	43.0	23.0	5.5	5.6	35.8
Basic slag.....	5.7	36.2	14.3	5.8	5.7	21.9	8.8	5.8	5.6	9.3
Superphosphate.....	4.8	80.0	42.8	6.1	6.2	75.6	15.4	5.9	5.8	56.0
Rock phosphate.....	5.2	40.8	—	—	6.1	17.2	—	—	5.6	32.1
Na ₂ HPO ₄ dosed.....	4.2	75.0	36.9	5.4	5.6	57.6	25.2	5.1	5.1	49.9
Na ₂ HPO ₄	4.9	50.0	—	—	5.7	32.8	—	—	5.2	33.3
Controls.....	2.9	66.6	88.5	3.7	3.2	96.1	87.6	3.7	3.3	90.8
Bonemcal "B".....	—	—	24.1	5.7	—	—	19.2	5.5	5.7	24.6

DISCUSSION OF RESULTS.

A study of the tables given above brings out a number of important points:—

(1) For both 1929-1930 and 1930-1931 the period during which increase in weight of the experimental animals took place was October to May. In fact October, 1931, to January, 1932, continued to show the anticipated increase. This observation is in accordance with general experience in Bechnanaland.

(2) The period May to October is one of food scarcity and consequently of practically no increase or even a drop in weight. This fact was verified for 1930 and 1931 and is usually borne out under farming conditions.

(3) The rock phosphate used is unsuitable as a phosphatic supplement. Table 1 shows a very poor increase for this group for the period October, 1929, to May, 1930. In fact the control group gained slightly more in weight and showed a smaller decrease in the successive winter period May to October, 1930, than the group receiving rock phosphate for the corresponding periods. Three groups, viz., those getting bonemeal "A", sodium phosphate in the water supply and those being dosed with sodium phosphate showed more than double the gain in weight for October, 1929, to May, 1930, than that of the rock phosphate group. For May to October, 1930, this latter group lost more weight than any other although it is closely followed by the superphosphate group. The inorganic phosphorus content of the blood indicates phosphorus sufficiency in the diet. Pica-testing revealed a very low percentage of depraved appetite—practically the lowest of all the groups—although it remains questionable whether this form of testing is a true reflection of the needs of the animals or of its idiosyncrasies at the time of testing. It may certainly be said that the members of the group in question show symptoms strongly resembling those observed by Du Toit *et al.* (1932) when studying the effect of sodium fluoride on pregnant heifers. The animals appeared unthrifty, listless and slow of movement. Hard knob-like swellings were noticed on the long bones of the front legs, which were swollen around the joints and the animals appeared disinclined to walk. The low percentage craving may therefore be due to the general state of inactivity of the animal rather than to the comparative absence of osteophagia. Furthermore, on post-mortem examination of five of these animals they were found to suffer very extensively from round-worm infection. In spite of the worm infection it would appear, however, that the clinical symptoms observed in this group when compared with those described in the paper by Du Toit *et al.*, already referred to, strongly suggest that the ultimate cause of the poor state of health of the rock phosphate group was due to a factor which was present in both experiments, namely, fluorine. Apart from this contention the symptoms agree very closely with those described by Hupka and Götze (1931) for fluorine poisoning in cattle, thus strengthening the belief that the extreme poor condition of the animals in the rock phosphate group was primarily due to the effects of the fluorine contained in and ingested with the phosphorus supplement. The fact must be stressed, therefore, that rock phosphate containing 3.5 per cent. of fluorine when given in quantities of 2 oz. or more per

day is detrimental to the health of bovines and is practically certain to cause death. The animals of the rock phosphate group were transferred to Onderstepoort for pathological study a year after the beginning of the experiment when the animals could hardly walk and were little more than skin and bones. They weighed on an average only 40 lb. heavier than at the beginning of the experiment and it was doubted whether they would stand the two day train journey successfully. This group was, therefore, eliminated from the experiment in October, 1930. The photographs given below show the animals after 12 months' supplementary feeding of rock phosphate as described above.





(4) Group 7 receiving sodium phosphate in its drinking water undoubtedly responded best to its treatment. For the period October, 1929, to May, 1930, each animal gained on an average 299 lb. or 39 lb. more than the next best group, and 70 lb. more than the third best group for the same period. It is true that group 7 had more grazing at its disposal than the other groups, but it is equally true that during summer even 20 acres is far more than the animal requires, while during winter the other groups were moved to better grazing, whereas group 7 was left in its original paddock. Furthermore, it may be noted that the weight increase for May-October, 1930, is not markedly different from that of some of the other groups, which, obviously, might have been the case with better winter grazing than in the other groups. Nevertheless, the groups would have been on a better basis for comparison had they all grazed in the same paddock. In other words, group 7, which received sodium phosphate in its drinking water, having grazed in a different paddock, which most probably did not have any appreciable effect on the results obtained, cannot be regarded as providing sufficient data for concluding definitely that feeding sodium phosphate via the water supply is more satisfactory than dosing bonemeal or some of the other forms of phosphorus tried in this experiment as the results seem to indicate. It may, however, be inferred that there is excellent reason for believing that giving water soluble sodium phosphate in the drinking water is an improvement upon the customary method of dosing bonemeal. It should be added that Bechuanaland is very rich in lime so that a consideration of the absence of calcium in sodium phosphate and, may be, the animal's need for it, is unnecessary. Furthermore, as far as is known there are no phosphorus-deficient areas in the Union that can be said to be calcium deficient as well. Hence the considera-

tion of a phosphatic supplement does not include as far as is known that of a calcium or any other supplement. To all intents and purposes sodium phosphate ought to be as efficacious for removing phosphorus deficiency as an equivalent amount of available phosphorus in bonemeal or other phosphate, and the results obtained in group 7 of this investigation strongly strengthens such a contention. If this view is correct an obvious advantage would be to select a water soluble phosphate that best suits the conditions of the experiment. Disodium phosphate causes the precipitation of phosphate in Bechuanaland water containing fair quantities of calcium and magnesium salts, whereas monosodium phosphate is sufficiently acid to keep all the phosphates in solution, although the drinking water, after its addition, is not acid to the taste. The use of a water soluble acid phosphate therefore eliminates the addition of an acid to the water—an important advantage under farming conditions. These contentions, together with the excellent results obtained in this provisional experiment on the suitability of adding disodium phosphate to the water supply of animals to combat aphosphorosis in South Africa, were responsible for beginning a new investigation with four groups of heifers on the practicability of the administration of water soluble phosphates via the drinking water of cattle and the suitability of such phosphates when compared with bonemeal and calcium phosphate the efficacy of which has been proved time and again. The new water soluble phosphate experiment has been going successfully for the last eight months and will be reported on in due course.

Before terminating the discussion of the water soluble phosphate group of the investigation reported here, attention should be directed to Table 2. A high average value in inorganic phosphorus in the blood indicates, of course, that the phosphorus is available in this form. The percentage pica or osteophagia for the periods given, viz., October, 1929, to May, 1930, and May to October, 1930, is not markedly different from that for bonemeal but much less than that for the calcium phosphate group.

(5) Groups 4 and 5, in which were given basic slag and superphosphate respectively, may be taken together as their results are similar and on the whole not as good as those of the groups not yet considered, although definitely superior to those of the control group. Table 1 indicates that for the periods given (*a*) these groups showed less gain during the growing periods—October to May—than the calcium phosphate group. It must be pointed out that for the period October, 1930, to May, 1931, the batch getting superphosphate showed the greatest increase in weight. Remarkable gain took place, but as the two lightest animals died during that period the figure representing the weight increase, viz., 356, is not a true one. However, the fact remains that for the period mentioned the superphosphate group did at least as well as the best group in the experiment. It is when the other periods and the total duration of the experiment are considered that the similarity between groups 4 and 5 becomes more apparent. (*b*) The percentage increase in weight for both groups is appreciably lower than that of the other groups. (*c*) The decrease in weight during the season of food scarcity is greater in groups 4 and 5 than in the calcium phosphate group. It appears that in this respect group 5 with its daily supplement of superphosphate is in-

ferior to group 4 receiving basic slag. The latter group, although lighter than the CaHPO_4 group at the end of the experiment appeared normal on examination, whereas the superphosphate group was definitely abnormal and showed a modified form of the clinical symptoms of the rock phosphate group, e.g. the joints of the legs were distinctly swollen, and knob-like swellings were visible on the long bones of the front legs; walking was obviously a painful process. The identity of these symptoms with those in the rock phosphate group has since been confirmed pathologically. On analysis the superphosphate was found to contain 1.5 per cent. fluorine, so that it appears that the group of animals being dosed with superphosphate have been suffering from chronic fluorine poisoning, which was apparently not severe enough to produce cessation of growth as in the rock phosphate group. Under these circumstances the use of superphosphate cannot be recommended as a phosphorus supplement for cattle when given in daily doses of 4 oz. or more. With regard to basic slag it may be said that daily doses of 5 oz. of this product are inferior to calcium phosphate in that the latter produced 178 per cent. gain while the former showed 146 per cent. on the average initial weight. The animals did not appear to suffer any ill effects after 27 months of basic slag feeding. From the figures presented in Tables 1 and 2 it seems that 5 oz. of this product produced better growth than 3 oz. The former dose also reduced pica from 36.2 per cent. over the first period of the experiment, October, 1929, to May, 1930, to 14 per cent. for the corresponding period of the following year. The phosphorus content of the blood is indicative of phosphorus sufficiency for all the periods of the experiment. The final weight in January, 1932, of the basic slag group is lower than that of group 1 (calcium phosphate supplement), but it is obviously much better than that of the control group. In view of the fact that the animals did not develop clinical symptoms, and that individual build and conformation of such a small group of half-bred animals may accidentally account for its comparatively lower weight, it is difficult to condemn the use of basic slag for the prevention of aphosphorosis, and it can only be said that apparently 4 oz. of this material daily reduced osteophagia at least as well as bonemeal, while the inorganic phosphorus in the blood is proof of the availability of its phosphorus. The case strongly in favour of or definitely against basic slag, however, is not finally proved, although with the number of other phosphatic products that are easier to work with and apparently more palatable to the animal while smaller doses, at least as efficacious as basic slag, are required, it is unlikely that basic slag will ever be a keen competitor in the market of phosphatic products for the remedy of aphosphorosis in stock.

(6) The remaining groups 1, 2, 3, 8 and 10, with their daily supplements of 1 oz. calcium phosphate, 3 oz. bonemeal "A", 2 oz. degelatinized boneflour, 1.5 oz. of sodium phosphate and 2 oz. bonemeal "B" respectively, are more or less on a par and will be considered together. Tables 1 and 2 again provide sufficient analyses of the results obtained for the justification of several conclusions. The animals in these groups increased rapidly in weight from the beginning of the experiment in October, 1929, until May, 1930. During the ensuing winter months (May to October) there was a steady decline followed by a second rapid rise for the period of

abundance (October to May) of the following year. It would appear from the differences in weight of each group for the two periods October to May, 1929-1930, and 1930-1931, that the smaller doses of products as given on page 678 are not enough for optimum growth or at all events that considerably better growth took place in some cases during the second period. However, a comparison with the figures for weight increase quoted by Du Toit and Bisschop (1930) for the same periods of growth for the years 1926-1927 and 1927-1928 makes the differences for the two successive periods in the groups under consideration certainly less significant. The figures referred to are interesting and will be quoted. The weights of all the heifers and tollies in the four breeds of cattle used in the breeding experiment were included in calculating the figures mentioned below. The weights registered just after weaning (October, 1926), were taken as the initial weights and the increases for the periods calculated for the same animals during the two periods October-May, 1926-27, and October-May, 1927-28 as given in Table 3.

TABLE 3.
Increase in weight (in lb.) for the periods given.

Breeds.	Oct.-May, 1926-27.		Oct.-May, 1927-28.			Oct.-May, 1929-30.	Oct.-May, 1930-31.
	Heifers.	Tollies.	Heifers.	Tollies.			
Africander (half-bred)	233	247	326	315		—	—
Frieslands (half-bred)	190	281	237	266	CaHPO ₄ Bonemeal A	205 260	289 299
Red Poll (half-bred)	240	271	279	307	Deg. bone- flour..... Na ₂ HPO ₄ ...	210 229	281 276
Sussex (half- bred).....	230	239	281	327		—	—
Averages.....	223	259	276	304		226	286

The weights for 1926-1928 are of animals receiving the daily 3 oz. dose of bonemeal. It seems from a consideration of the weight increases that a difference for two successive periods (October-May) of about 50 lb. is not uncommon for a group of tollies or heifers so that the differences in weight increase for the periods in question and for the groups under consideration in this publication become less significant. In some cases, however, the differences in weight between the two periods are quite considerable, e.g. the groups receiving CaHPO₄ and degelatinized boneflour respectively, and it seems probable that better results would have been obtained with larger doses for October, 1929, to May, 1930.

The dose of sodium phosphate was not increased for the period October, 1930, to May, 1931, and yet this group also showed a considerably greater increase for the latter period when compared with that of October, 1929 to May, 1930.

The groups 1, 2, 3, 8 and 10 show little difference and apparently all the materials in question are excellently suitable for the prevention of aphosphorosis. Table 2 bears out this view for the high values for inorganic phosphorus in the blood of the animals of all the groups indicate sufficient availability of phosphorus in the materials used. It must be pointed out that osteophagia or the percentage pica is unsatisfactory in most of the groups. One fact stands out clearly, viz., that pica is much higher in the control group than in any of the others. This observation is a fact well known to farmers. The nature of the test, i.e., giving each animal free access to a box of rotten bones and a box of sweet bones once a fortnight and recording whether such an animal picks up a rotten or a sweet bone, is such that it does not lend itself to the grading of cravers as the percentage craving expressed in Table 2 might cause one to infer. For general purposes of distinguishing between cravers and non-cravers, the test is sufficient. In Table 2 sweet bone and rotten bone cravers have been grouped together as cravers and the figure given represents the percentage number of times such craving was recorded at the tests. Three animals died in the control group of lamsiekte during the course of the investigations, whereas no other animals contracted this disease.

Blood analysis for phosphorus is indicative of the phosphorus equilibrium of the animal and it can safely be said that the figures obtained for inorganic phosphorus do not suggest aphosphorosis in any of the groups except the controls, although the craving, if it is associated with phosphorus deficiency only, still suggests the presence of deficiency in some of the groups. Results of pica-testing should be interpreted on general lines as indicated above, and its presence not necessarily associated with aphosphorosis. Even bonemeal which usually reduces pica to a minimum was less satisfactory in that respect in this investigation. The groups receiving calcium phosphate, superphosphate and sodium phosphate, apart from the control group, which is the extreme, of course, all showed rather high osteophagia.

Before leaving the groups under discussion it would be well to glance at Figures V and VI giving the weight curves of the groups receiving bonemeal compared with the control group on the one hand and the calcium phosphate group on the other. The groups receiving bonemeal undoubtedly show an advantage over the calcium phosphate group and it appears that even one ounce of calcium phosphate per day does not produce quite as rapid growth in bovines as 3 ounces of bonemeal. This observation agrees with that made by Du Toit and Green in 1930 for $\frac{2}{3}$ oz. CaHPO_4 when compared with 3 oz. of bonemeal.

In conclusion it may be pointed out that from May to October there was a slight decrease in weight in all the groups. The control group with no supplement lost only slightly more weight than some of the other groups, e.g. bonemeal group lost 24 lb. from May, 1930, until October, 1930, while the control group lost 31 lb. for the same period. The difference was greater in some of the other cases but omitting the rock phosphate, superphosphate and basic slag groups, the differences in the decreases for the periods (May-October) are not very significant. It must be remembered that the decrease took place

in spite of the supplements that were given regularly and that the control group was only slightly worse off with no supplement at all. The idea suggests itself, therefore, that it may be more economical to feed phosphatic supplements only during the natural period of growth of cattle, i.e. October to May, in phosphorus-deficient areas where no lamsiekte occurs. This would mean that during the winter months when the animals can least afford the daily drives to the crushes they would be spared the wastage of energy that the daily drive incurs at the cost of their daily ration of supplement, of course. The possibility always remains that, even if animals only on pasture lose slightly more weight than others getting a bonemeal supplement, they might increase in weight more rapidly than the latter during the following summer season if given enough phosphatic supplement for optimum growth. Obviously this argument does not apply to lamsiekte areas where the cessation of phosphorus feeding from May to October would naturally result in an increase of pica leading to losses through lamsiekte.

Lusk *et al.* (1930) found supplementary feeding during the winter period of loss in weight uneconomical, for the animals did not attain bigger weights ultimately than those that had not been fed in the period of scarcity, while the latter group invariably showed a greater rate of increase during the following period of abundance. This idea of normal phosphorus feeding during summer and withholding such supplements during the winter months will be tested out in the near future against the general practice of feeding equal quantities of phosphatic supplements all the year round. Bekker (1932) advocates feeding smaller quantities of phosphorus during winter, when no weight increase takes place, i.e. to feed for maintenance only.

SUMMARY.

(1) Precipitated calcium phosphate, two brands of bonemeal, degelatinized boneflour, basic slag, superphosphate, ground Egyptian rock phosphate, sodium phosphate and sodium phosphate in the drinking water, have been given to groups of cattle for periods varying from 12 to 27 months.

(2) Rock phosphate was found to be unsuitable as a supplement for supplying phosphorus to cattle. Growth was poor and practically ceased after 12 months, when the animals were only 40 lb. heavier than at the beginning of the investigation. Symptoms of poisoning appeared soon after the start and gradually became worse until the animals were in a pitiable state 12 months after the beginning of the experiment, when the group had to be eliminated.

(3) The group receiving its daily dose of superphosphate responded well from the beginning, remained throughout in a better condition than the controls and showed a very fair increase at the end of the period. Clinical symptoms not unlike those of the group receiving rock phosphate developed towards the end of the experiment and it was found that 1.5 per cent. fluorine was present in the product. The suggestion is put forward that these animals, like those in the rock phosphate group, were suffering from fluorine poisoning.

(4) The weight increase of the animals dosed with basic slag is at least as good as those in the superphosphate group. Apparently 5 oz. basic slag containing 23.9 gm. P_2O_5 do not give better results as a daily dose than one of calcium phosphate containing only 10.7 gm. P_2O_5 .

(5) The results obtained with daily doses of 8.6 gm. P_2O_5 given as sodium phosphate, both as the dry compound, and when given in the drinking-water, with 10.7 gm. P_2O_5 as calcium phosphate, 16.7 gm. P_2O_5 as the two brands of bonemeal used, and with 16.7 gm. P_2O_5 as degelatinized boneflour are very satisfactory and agree closely with normal increases in weight for this class of stock when bonemeal is fed. Both brands of bonemeal hold a slight but definite advantage over the other supplements. Considering the sizes of the daily doses given it would appear that the products could be placed in the following order of availability of phosphorus:—

1. Sodium phosphate.
2. Precipitated calcium phosphate.
3. Bonemeal and degelatinized boneflour.

(6) Giving disodium phosphate in the drinking water of cattle yields good results and has a number of advantages over the method of dosing daily.

(7) Blood analyses for inorganic phosphorus revealed phosphorus sufficiency in the groups receiving the supplements and deficiency in the controls.

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REFERENCES.

- BEKKER, J. G. (1932). The Administration of Phosphorus to Animals through their Water Supply. This Report.
- DU TOIT, P. J., AND GREEN, H. H. (1930). Dicalcium Phosphate as a Source of Phosphorus in Nutrition. *16th Rept. Dir. Vet. Serv. and A.I.*, p. 267-287.
- DU TOIT, P. J., AND BISSCHOP, J. H. R. (1929). The Breeding of Cattle on Phosphorus Deficient Veld. *15th Rept. Dir. of Vet. Serv.*, p. 1059-1169.
- DU TOIT, P. J.; MALAN, I. A.; GROENEWALD, J. W.; AND DE KOCK, G. (1932). The Effect of Fluorine on Pregnant Heifers. This report.
- LUSK, J. L., *et al.* (1930). The Normal Growth of Range Cattle. Bull. 409, College Station, Brazos, Country Texas.
- OTTO, J. S. (1932). Balance Experiments with Phosphates. This Report.
- THEILER, A.; GREEN, H. H.; AND DU TOIT, P. J. (1924). Phosphorus in the Live Stock Industry. *Int. Dept. Agric.*, U. of S.A., May, 1924.
- THEILER, A.; GREEN, H. H.; AND DU TOIT, P. J. (1928). Studies in Mineral Metabolism III. Breeding of Cattle on Phosphorus Deficient Pasture. *Int. Agric. Sc.*, Vol. 18, Part 3, pp. 369-371.
- THEILER, A.; VILJOEN, P. R.; GREEN, H. H.; DU TOIT, P. J.; MEIER, A.; AND ROBINSON, E. (1927). Lamsiekte (Parabotulism) in Cattle in South Africa. *11th and 12th Rept. of Dir. of Vet. Educ. and Res.*, Part 2, p. 1233.

APPENDIX.

PERCENTAGE PICA. GROUP AVERAGE.

Groups.	1929.			1930.											
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
1. $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	—	92.5	70.8	98.7	93.7	93.7	75.0	81.2	47.6	42.9	71.4	71.4	42.9	71.4	42.9
2. Bone meal, "A".....	—	37.5	50.0	29.5	50.0	31.3	31.3	50.0	28.5	4.3	35.7	35.7	21.5	28.6	33.3
3. Deg. Bone flour.....	—	35.6	38.1	21.4	42.8	28.5	28.5	42.8	21.4	28.5	28.5	21.4	28.6	14.3	22.6
4. Basic slag.....	—	58.3	66.6	83.5	91.7	100.0	83.3	83.3	91.4	83.5	75.0	83.3	24.9	45.5	73.3
5. Superphosphate.....	—	50.0	31.3	12.5	43.7	50.0	56.3	50.0	16.6	18.7	12.5	25.0	—	—	—
6. Rock phosphate.....	—	31.3	70.8	87.5	87.5	75.0	56.3	87.5	61.8	57.1	57.1	50.0	28.6	50.0	47.6
7. NaHPO_4 dosed.....	—	43.7	50.0	56.3	56.3	50.0	56.3	37.5	52.3	28.5	42.8	14.3	100.0	100.0	26.6
8. NaHPO_4 water.....	—	31.5	56.5	50.0	100.0	83.3	83.3	83.3	100.0	100.0	28.6	100.0	21.5	21.5	40.5
9. Controls.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10. Bone meal "B".....	—	—	—	—	—	—	—	—	19.1	21.5	28.6	35.7	21.5	21.5	—

Groups.	PERCENTAGE PICA. GROUP AVERAGE.												1932.
1931.												Jan.	
	Jan.	Feb.	Mar.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
1. $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	71.4	42.9	78.6	42.9	28.6	35.7	14.3	35.7	35.7	50.0	23.8	71.4	57.1
2. Bone meal, "A".....	33.7	28.6	35.7	14.3	14.3	21.5	14.3	14.3	28.6	14.3	19.1	28.6	57.1
3. Deg. Bone flour.....	57.1	21.4	35.7	42.9	—	23.8	21.4	42.9	28.6	21.4	19.1	21.5	28.6
4. Basic slag.....	—	21.4	28.6	14.3	—	—	—	35.7	21.4	—	14.3	28.6	14.3
5. Superphosphate.....	—	25.0	37.5	25.0	25.0	25.0	—	25.0	75.0	25.0	25.0	—	25.0
6. Rock phosphate.....	—	—	—	—	—	—	—	—	—	—	—	—	—
7. NaHPO_4 dosed.....	35.7	28.6	50.0	21.5	28.6	23.9	21.5	35.7	33.7	14.3	42.8	21.5	50.0
8. NaHPO_4 water.....	—	—	—	—	—	—	—	—	—	—	—	—	—
9. Controls.....	100.0	90.0	90.0	70.0	70.0	93.3	90.0	100.0	100.0	70.0	86.1	100.0	100.0
10. Bone meal "B".....	33.3	33.3	41.6	16.7	16.7	16.8	50.0	33.3	25.0	25.0	25.0	33.3	—

GROUP AVERAGE.

INORGANIC PHOSPHORUS.

Group.			1929.				1930.												1931.							
			Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sept.
1.	CaHPO ₄	—	3.2	5.8	5.1	5.2	4.7	3.8	4.6	6.1	6.3	6.1	6.0	5.5	6.3	5.5	4.6	7.3	5.3	5.2	5.7	5.6	5.3	5.7	6.6	5.6
2.	Bone meal "A".....	—	3.1	5.9	5.3	5.3	5.5	4.3	5.4	5.9	6.3	6.1	5.6	5.5	6.9	5.9	4.7	7.0	5.6	6.4	5.8	6.1	5.5	6.1	5.2	5.0
3.	Deg. Bone flour.....	—	3.2	6.8	6.8	5.3	5.2	4.1	4.9	5.9	7.8	6.1	4.6	6.1	6.2	5.0	4.4	7.0	5.0	6.1	4.5	5.8	5.6	5.6	6.5	5.1
4.	Basic slag..	—	3.5	6.7	6.2	5.4	5.3	4.7	5.3	6.1	6.9	5.8	5.5	4.8	5.6	6.3	5.5	6.5	5.4	6.1	6.4	5.4	5.9	5.5	6.5	5.5
5.	Superphos- phate.....	—	3.2	5.5	6.8	4.7	4.6	4.2	4.5	6.7	6.9	6.5	6.4	6.3	6.8	6.1	5.0	7.6	6.4	5.6	4.9	6.5	6.1	6.2	6.3	5.6
6.	Rock phos- phate.....	—	3.1	6.8	7.1	5.1	4.7	5.1	5.2	6.1	5.9	6.4	5.9	—	—	—	—	—	—	—	—	—	—	—	—	—
7.	NaHPO ₄ dosed.....	—	3.1	4.7	4.5	4.3	5.1	3.6	4.6	5.4	7.7	5.9	5.4	5.4	6.8	5.5	4.7	6.7	4.8	5.3	4.2	5.1	5.6	5.0	5.7	5.1
8.	NaHPO ₄ water.....	—	2.9	6.4	6.7	5.2	4.6	4.6	4.0	5.6	7.5	5.6	5.6	5.7	6.7	4.6	—	—	—	—	—	—	—	—	—	—
9.	Controls.....	—	3.2	3.7	3.1	2.8	2.8	2.8	2.5	3.3	3.7	2.9	2.6	4.6	3.8	2.3	2.8	5.7	4.0	3.2	3.9	3.5	3.6	3.7	3.7	3.9
10.	Bone meal "B".....	—	—	—	—	—	—	—	—	—	5.9	7.0	5.8	5.8	5.7	6.2	6.1	4.3	7.0	5.7	5.6	5.8	5.7	5.2	5.9	4.9

Fig. 1.—Weight Curves.

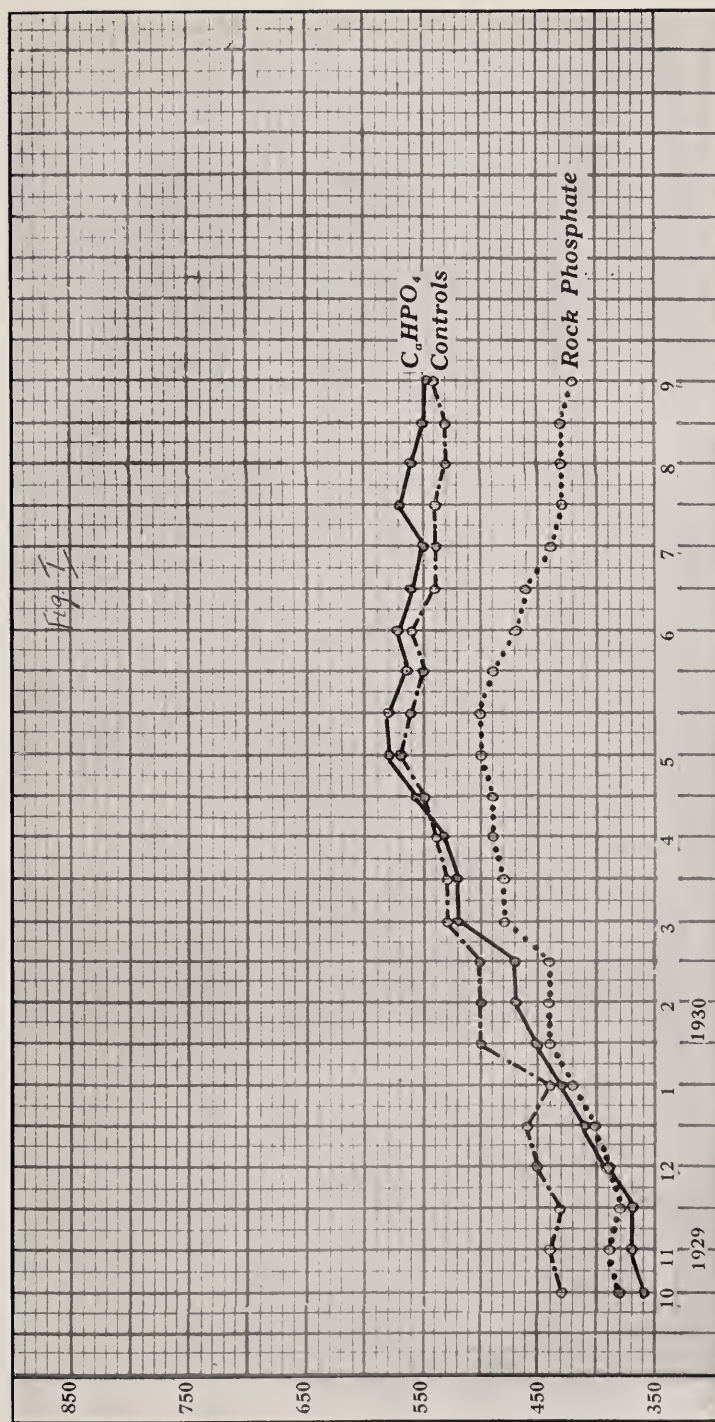


Fig. 2.—Weight Curves.

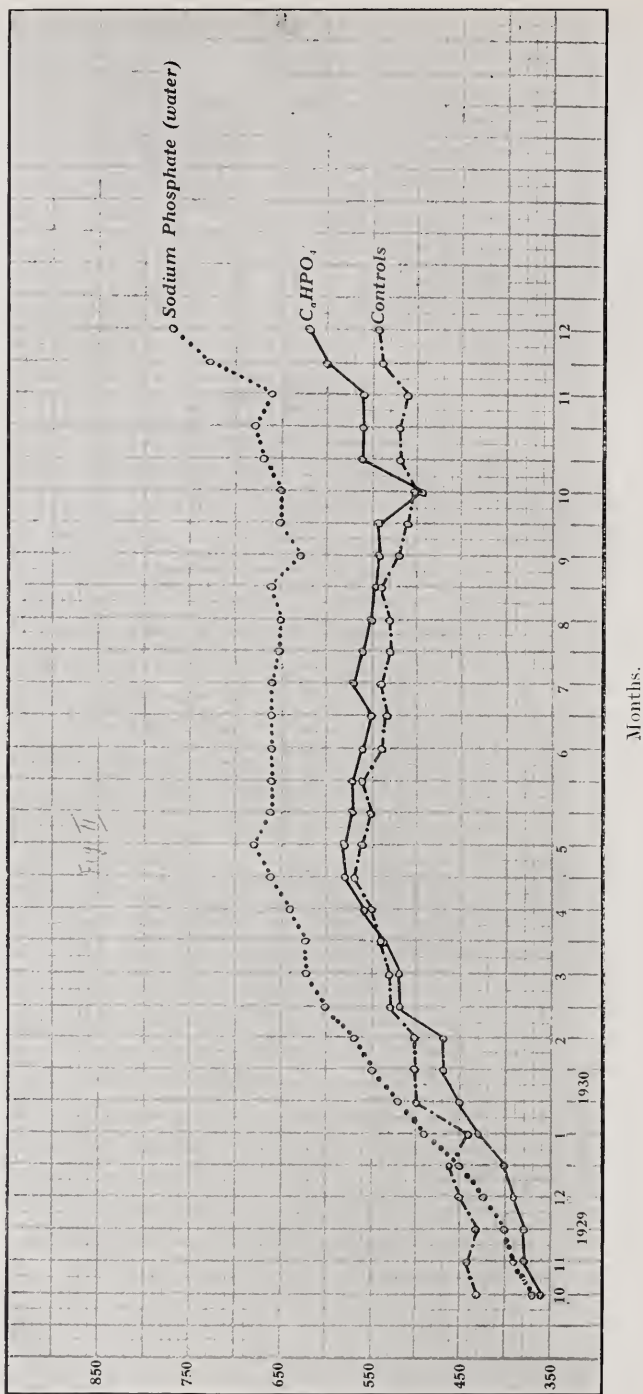
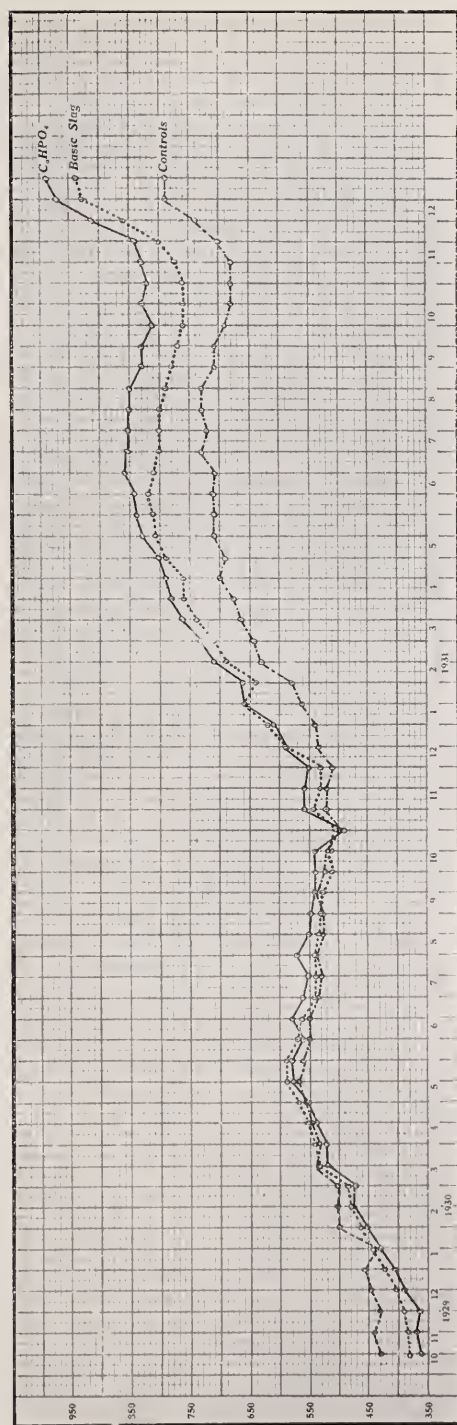
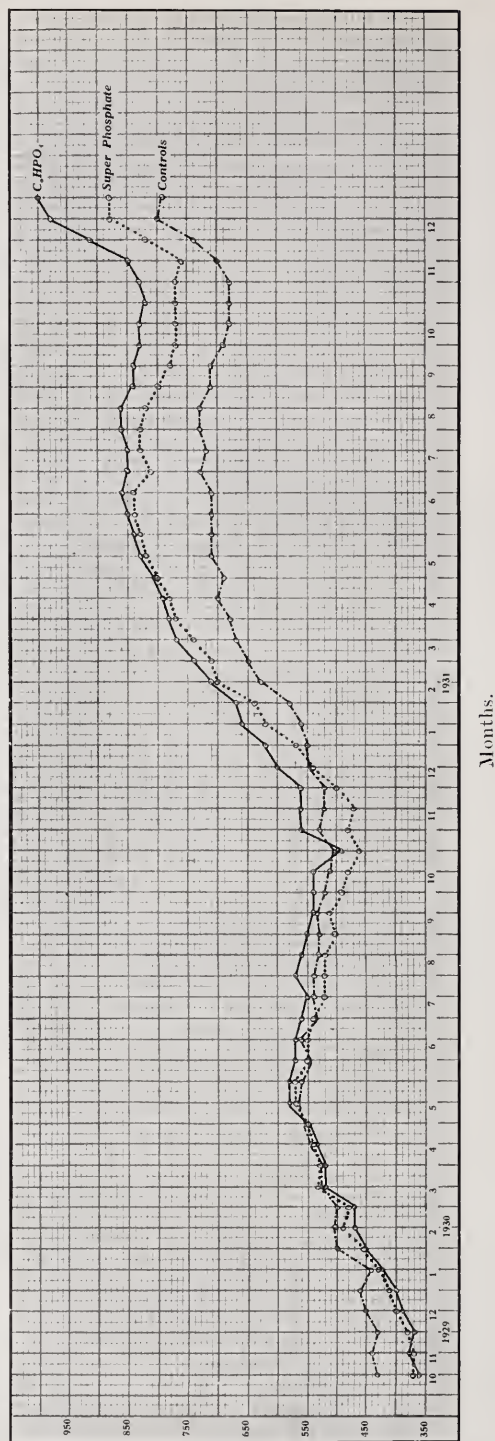


Fig. 3.—Weight Curves.



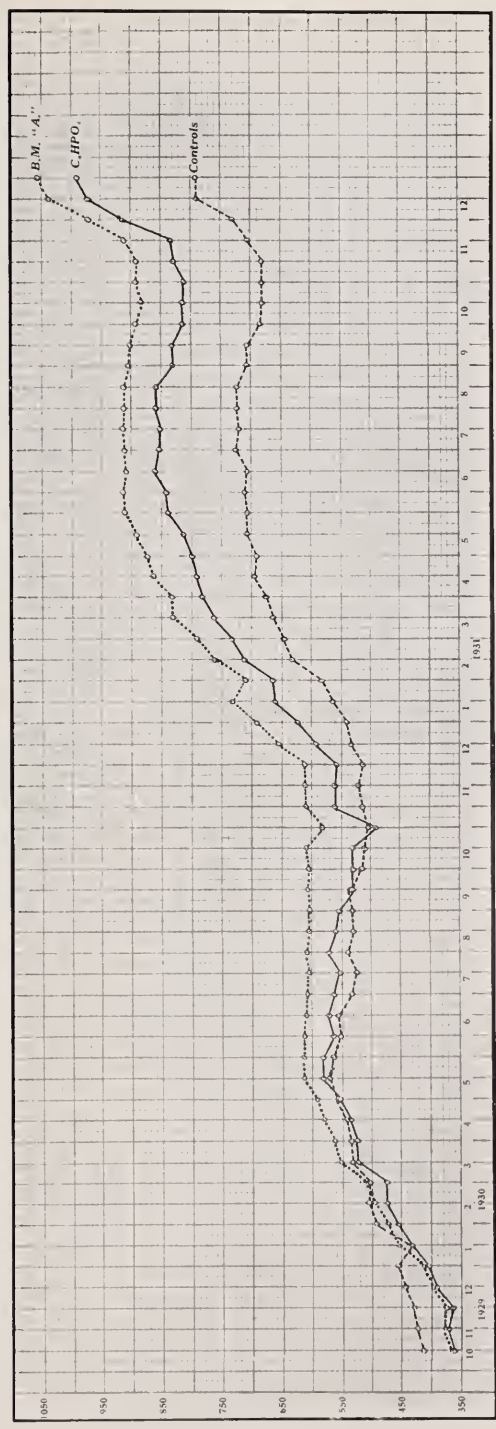
Months.

Fig. 4.—Weight Curves.



Months.

Fig. 5.—Weight Curves.



Months.

Fig. 6.—Weight Curves.

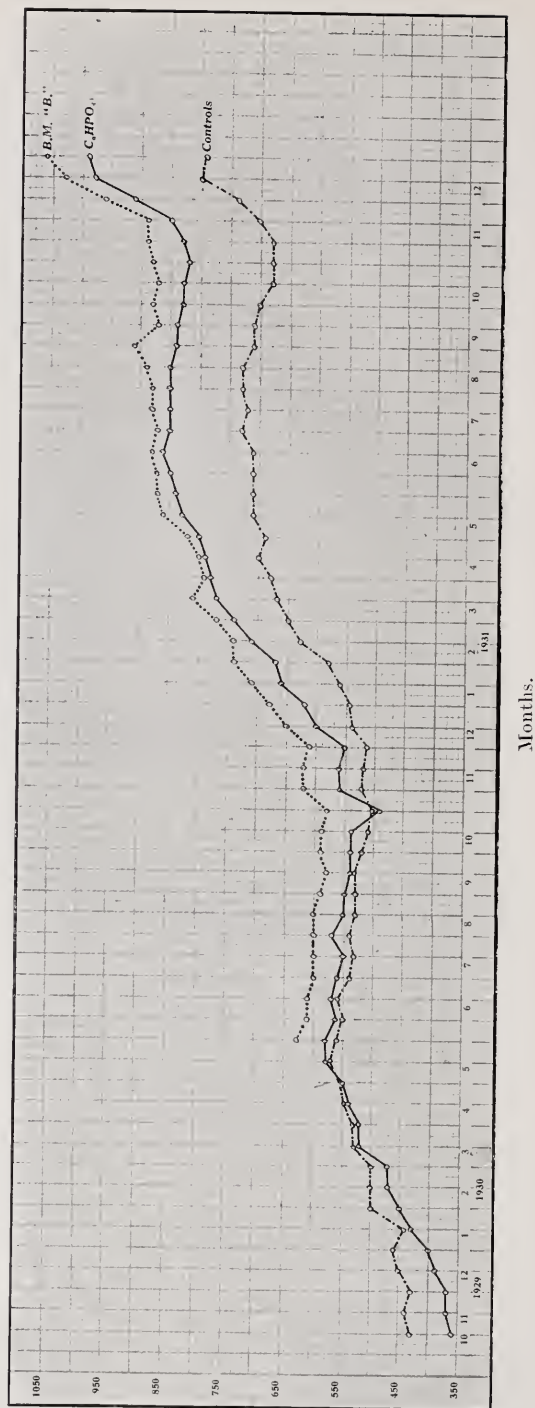
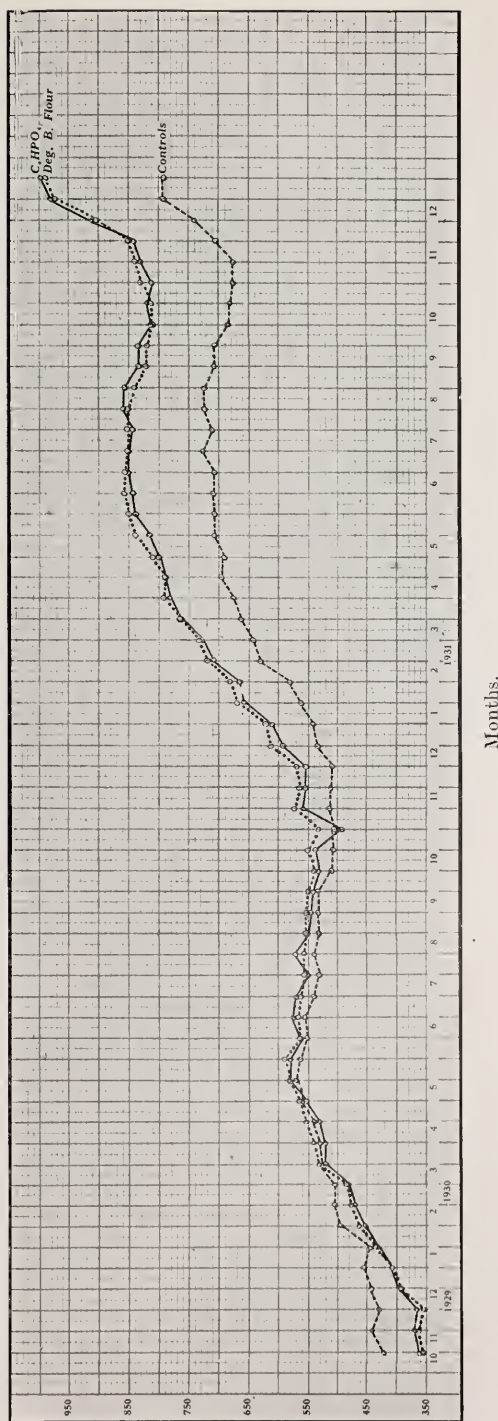
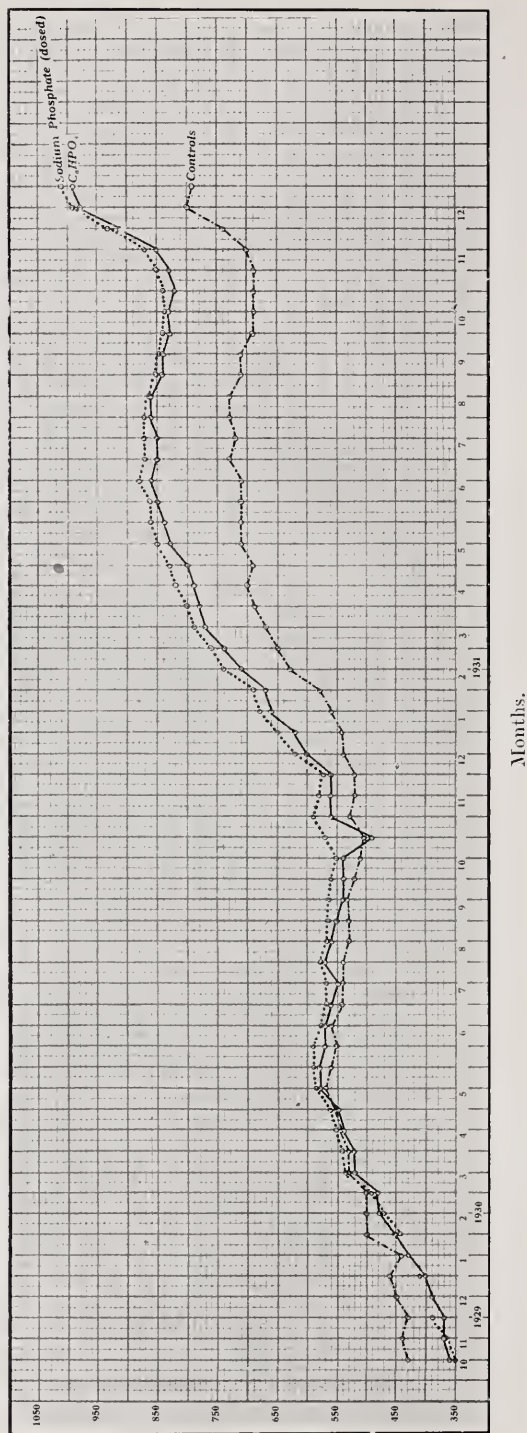


Fig. 7.—Weight Curves.



Months.

Fig. 8.—Weight Curves.



Studies in Mineral Metabolism XXII: Phosphorus, Calcium and Protein.

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INTRODUCTION.

A NOTEWORTHY feature of South African pastures and soils is their low phosphorus content, as shown by Juritz (1909), Theiler *et al.* (1915, 1927), van Zyl (1928), and Henrici (1928, 1930).

Theiler, Green and du Toit (1924) comment on the low P_2O_5 content of South African pastures, averaging in some cases only .08 per cent. P_2O_5 , or only one-fifth of that present in ordinary European hay. These authors (1927) express the view that over wide areas of the Union of South Africa, the level of the phosphorus reached in the natural vegetation is below the physiological optimum requirements of stock. Supplementary feeding of phosphates to supply what is deficient in the pastures has been shown to lead to the elimination of aphosphorosis and diseases associated with it and, moreover, to lead to increased production.

Such supplementary feeding has, therefore, become the rule in most areas and an essentiality in others where phosphorus deficiency was so acute that farming with cattle was unprofitable. Of the commercial phosphatic products used for stock feeding in this country to-day the advantage still lies with bonemeal, as it was the first to be recommended to stock farmers, is cheap, and easily procurable, but it is an open question whether bonemeal is the best and most economical supplement.

Theiler *et al.* (1920, 1924, 1927) in their phosphorus deficiency experiments fed 3 oz. of bonemeal per head per day to growing cattle, but du Toit and Green (1930) concluded that this dose was probably in excess of the requirements of the animal for optimum growth and 1 oz. definitely below.

However, it was thought that balance experiments with phosphatic products amongst which bonemeal would be included, would throw further light upon the phosphorus requirements of animals from a different angle. In the past, conclusions with regard to this matter have been based upon the weight increase of groups of animals over extended periods, whereas in this investigation maximum absorption was aimed at and the quantities of supplements therefore varied according to the results of the analysis of the feed and the excreta respectively. Furthermore, as calcium metabolism

is probably closely associated with that of phosphorus, the investigation to be reported on in this publication concerns itself, in the first instance, with the following matters:—

(1) The availability of phosphorus in several phosphatic products on the market. This work was undertaken with a view to its direct application in practical farming and the materials containing phosphorus selected accordingly.

(2) The effect of variations in the Ca-P ratio in the ration upon the absorption of those two constituents.

(3) A study of the effect of phosphorus and calcium in the feed upon the consumption and utilization of the latter. The ingestion and absorption of proteins, carbohydrates, fats and fibre are involved in this work.

EXPERIMENTAL WORK.

A. THE ANIMALS.

Two Friesland Grade animals were used. Ox No. 2696 weighed 1,050 lb. and was 33 months old in September, 1930, when the investigation began. Heifer No. 3654 weighed 700 lb. at the age of 20 months when she was placed in the metabolism experiment in September, 1930.

B. THE METABOLISM STALLS.

Two metabolism stalls 12 by 8 by 10 feet with concrete walls and floors were available. The floor slopes slightly towards a central hole fitted with a grate, underneath which—in the basement, therefore—a bucket with a muslin cover is placed. Small pieces of faeces are thus prevented from dropping into the bucket in which the urine is collected. A long adjustable pole divides the stable lengthwise into two, and the animal occupies one part at a time to facilitate the periodic collection of the faeces, in the unoccupied portion. This arrangement allows the animal 12 by 4 ft. to move about without interference and worked excellently throughout the course of the investigation. Meigs, Blatherwick and Cary (1919) concluded from their results that the disturbance of the animal due to the collection of faeces and urine in a metabolism experiment interfered with the assimilation of phosphorus, nitrogen and more especially calcium.

C. SAMPLES OF FAECES AND URINE FOR ANALYSIS.

The total faeces passed in a 24-hour period is weighed, then thoroughly mixed by hand and exactly 1,000 gm. taken. This quantity is spread uniformly on a piece of brown paper of known weight and dried in a water-bath or gas-oven. The faeces when dry, is taken out of the oven and left exposed overnight at room temperature and then weighed. It is ground in a mill and sampled for analysis. The 24-hour collection of urine is measured, thoroughly mixed and a sample taken. The analysis of the urine is done immediately after collection. The faeces and urine are collected daily at 7.30 a.m.

D. WEIGHING.

The animals were weighed several times each week at 8.30 a.m. on a weighbridge about 50 yards from the metabolism stables. They were allowed a free run in a small paddock next to the weighbridge for 15 minutes once a week, when they were also brushed down. No difficulty was encountered in preventing the animals from urinating and defecating while outside the metabolism stalls.

E. FEEDING.

It was difficult to obtain a suitable basal ration, for while the animals had to be fed suitably for growth, the ration had to be sufficiently poor to be deficient in phosphorus and, if possible, in calcium, so that supplementary feeding of either did not necessarily mean that excess of the constituent in question was being given, and furthermore, so that investigations could be carried out under conditions of mineral deficiency, sufficiency and excess, if needs be. After some experimentation the following basal ration was decided upon. A minimum quantity of poor quality hay to keep the ingestion of Ca and P as low as possible, and yet provide for the requirements of roughage of the animal, yellow crushed maize and rolled flaked maize, or fanko, very low in Ca and P were given. The quality of the protein was improved by the addition of a small quantity of aleuronat, a protein concentrate prepared from seeds. About an ounce of common salt was given daily. The approximate composition of the daily ration is given in the following table:—

DAILY RATION.

Feeding Stuff.	Amount given		P ₂ O ₅ .	CaO.	Protein.	Net Energy. Value, Therms.
	Ox 2696.	Heifer 3654.				
						Per 100 lb. dry matter.
Hay.....	4.0 Kg.	3.0 Kg.	.25 %	.45 %	5.0 %	20
Maize.....	2.5 Kg.	2.5 Kg.	.52 %	.025 %	8.8 %	85
Fanko.....	3.0 Kg.	2.5 Kg.	.09 %	.01 %	7.8 %	85
Aleuronat.....	20 gm.	20 gm.	—	—	7.7 %	—
Sod. Chloride...	30 gm.	25 gm.	—	—	—	—
Water.....	<i>ad lib.</i>	<i>ad lib.</i>	—	68 mgm. per litre	—	—

METHOD OF ANALYSIS.

The methods adopted for calcium and phosphorus determinations are those described by Malan and van der Linghe (1931). For the determination of calcium in urine, 20 c.c. of urine are dried and ashed. The ash is dissolved in 20 c.c. 10 per cent. hydrochloric acid, washed into a 100 c.c. flask and water added to the mark. Some of this solution is then filtered through Whatman No. 40 paper and a suitable aliquot 2.5 c.c. taken for each determination. The procedure described by the authors mentioned above is then followed. In order to determine the phosphorus in the urine, 2 c.c. urine and

1 c.c. concentrated sulphuric acid are combusted in a pyrex test tube. The contents of the tube are boiled over a flame in a fume cupboard until all the water is driven off, then 30 per cent. perhydrol, $\frac{1}{2}$ c.c. at a time, is added and the solution boiled until clear.

When cool the contents of the tube are diluted with about 7 c.c. water, and neutralised with strong ammonia. The solution is then washed into a 100 c.c. cylinder and phosphorus determined in an aliquot according to the method for grass extracts described by Malan and van der Lingen, already mentioned.

The fibre, ether extract, nitrogen (protein) and ash were determined according to the methods described by Wood (1911).

EXPERIMENT 1.

THE AVAILABILITY OF PHOSPHORUS IN BONEMEAL.

Duration of Experiment: 10.10.30-28.10.30.

The animals were placed in the metabolism stables and kept on the basal ration plus 70 gm. calcium carbonate several weeks prior to the actual start of the experiment. During this period no supplement of phosphorus was given, so that the diet was phosphorus deficient.

For the last four days of this preliminary period the excreta was collected for analysis. Then 90 gm. bonemeal, i.e. 22.5 gm. P_2O_5 were added to the ration daily and the calcium carbonate decreased to retain a constant intake of calcium.

The experimental period, i.e. during which the bonemeal was given, lasted for 7 days, then a period of 7 days followed, during which the animals were again placed on the ration of the pre-period. On the last day of each period the animals were bled and the inorganic phosphorus of the blood determined as described by Green (1928).

	Bovine.	Pre-liminary Period.	Experimental Period.	After Period.
Inorganic phosphorus in mgm. per 100 c.c. blood.....	2696	4.25	7.5	5.15
	3654	5.27	7.8	4.87

The figures indicate that high inorganic phosphorus in the blood is associated with high phosphorus in the ration. This was shown to be the case by Malan, Green and du Toit (1928) when working with cattle under veld conditions. The calcium and phosphorus balances are given in the following tables:—

TABLE 1.—EXPERIMENT 1.

P₂O₅ BALANCE OF BOVINE 2696: BONEMEAL FED.

24-hour Period.	Intake P ₂ O ₅ gm.	Outgo P ₂ O ₅ gm.		Absorption P ₂ O ₅ gm.	Retention P ₂ O ₅ gm.	Phosphate Supplement.
		Faeces.	Urine.			
1	19.40	12.60	.111	6.80	6.69	0.
2	19.63	13.90	.112	5.73	5.62	0.
3	19.43	10.98	.110	8.45	8.34	0.
4	19.12	11.39	.114	7.73	7.62	0.
Average...	—	—	—	7.18	7.07	0.
5	41.77	14.30	.113	27.47	27.36	22.5 gm. P ₂ O ₅ as B.B. bonemeal.
6	41.14	12.92	.130	28.22	28.09	" "
7	42.01	16.50	.390	26.51	26.12	" "
8	41.68	16.60	.687	25.08	24.39	" "
9	41.91	16.48	1.400	25.43	24.03	" "
10	42.15	18.21	.920	23.94	23.02	" "
11	42.25	19.37	.465	22.88	22.41	" "
Average for periods 8-11...	—	—	—	24.33	23.46	0.
12	20.02	13.57	.347	6.45	6.10	0.
13	20.02	12.46	.258	7.56	7.30	0.
14	20.04	12.45	.260	7.59	7.33	0.
15	20.04	12.30	.173	7.74	7.57	0.
16	20.04	13.20	.212	6.84	6.63	0.
17	20.04	13.36	.116	6.68	6.56	0.
18	20.04	14.50	.114	5.54	5.43	0.
Average for periods 15-18...	—	—	—	6.70	6.55	0.

TABLE 2.—EXPERIMENT 1.

P₂O₅ BALANCE OF BOVINE 3654: BONEMEAL FED.

24-hour Period.	Intake P ₂ O ₅ gm.	Outgo P ₂ O ₅ gm.		Absorption P ₂ O ₅ gm.	Retention P ₂ O ₅ gm.	Phosphate Supplement.
		Faeces.	Urine.			
1	17.09	13.32	.164	3.77	3.61	0.
2	16.98	12.81	.149	4.17	4.02	0.
3	16.93	11.80	.148	5.13	4.98	0.
4	17.05	10.25	.152	6.80	6.65	0.
Average...	—	—	—	4.97	4.82	0.
5	42.18	12.15	.220	30.03	29.81	22.5 gm. P ₂ O ₅ as B.B. bonemeal.
6	43.52	15.30	1.260	28.22	26.96	" "
7	43.60	19.62	5.105	23.98	18.87	" "
8	43.35	24.60	5.775	18.75	18.97	" "
9	43.26	20.25	4.900	23.01	18.11	" "
10	43.15	23.24	6.320	19.91	13.59	" "
11	42.93	23.40	5.660	19.53	13.87	" "
Average for periods 8-11...	—	—	—	20.31	14.64	0.
12	20.63	20.35	3.330	.28	3.05	0.
13	20.96	14.73	.768	6.23	5.46	0.
14	20.42	14.52	.281	5.90	5.62	0.
15	20.24	13.07	.244	7.17	6.93	0.
16	20.56	15.70	.326	4.86	4.53	0.
17	20.76	15.82	.314	4.94	4.63	0.
18	20.45	15.27	.229	5.18	4.95	0.
Average for periods 15-18...	—	—	—	5.54	5.26	0.

PHOSPHORUS, CALCIUM AND PROTEIN METABOLISM.

TABLE 3.—EXPERIMENT 1A.

CaO BALANCE OF BOVINE 2696: BONEMEAL FED.

24-hour Period.	Intake CaO gm.	Outgo ^a CaO gm.		Absorption CaO gm.	Retention CaO gm.	Phosphate Supplement.
		Faeces.	Urine.			
1	48.61	40.21	1.25	8.4	7.15	O.
2	49.22	49.5	1.26	— .28	— 1.54	O.
3	48.55	35.1	1.31	13.45	12.14	O.
4	48.31	37.1	1.60	11.21	9.61	O.
Average...	—	—	—	8.20	6.85	
5	48.34	42.3	1.32	6.04	5.72	22.5 gm. P ₂ O ₅ as B.B. bonemeal.
6	47.02	35.6	.61	11.42	10.81	" "
7	48.75	39.1	.76	9.65	8.89	" "
8	48.56	41.1	.44	7.46	7.02	" "
9	48.68	37.0	.51	11.68	11.17	" "
10	49.12	41.1	.63	8.02	7.39	" "
11	49.05	26.55	.53	22.50	21.97	" "
Average for periods 8-11...	—	—	—	12.42	11.89	
12	48.94	19.5	.98	29.44	28.46	O.
13	48.98	19.6	1.07	29.38	28.31	O.
14	48.77	22.2	.85	26.57	25.72	O.
15	49.11	21.85	.82	27.26	26.44	O.
16	49.03	28.4	1.24	20.63	19.39	O.
17	48.99	32.7	.55	16.29	15.74	O.
18	49.00	41.1	.90	7.90	7.0	O.
Average for periods 15-18...	—	—	—	18.02	17.14	

TABLE 4.—EXPERIMENT 1.

CaO BALANCE BOVINE 3654: BONEMEAL FED.

24-hour Period.	Intake CaO gm.	Outgo CaO gm.		Absorption CaO gm.	Retention CaO gm.	Phosphate Supplement.
		Faeces.	Urine.			
1	45.84	29.90	1.50	15.94	14.44	O.
2	45.14	39.10	1.69	6.04	4.35	O.
3	45.07	36.81	1.75	8.26	6.51	O.
4	45.33	30.58	1.35	14.75	13.40	O.
Average...	—	—	—	11.25	9.68	
5	44.13	33.30	1.21	10.83	9.62	22.5 gm. P ₂ O ₅ as B.B. bonemeal.
6	44.96	41.80	.70	3.16	2.46	" "
7	45.13	41.10	.63	4.03	3.40	" "
8	45.00	43.00	.49	2.00	1.51	" "
9	44.78	30.21	.71	14.57	13.86	" "
10	45.04	28.86	.75	16.18	15.43	" "
11	44.60	32.12	.71	12.48	11.77	" "
Average for periods 8-11...	—	—	—	11.31	10.64	
12	45.56	32.57	.53	12.99	12.46	O.
13	46.03	24.40	.68	21.63	20.95	O.
14	45.47	25.27	.49	20.20	19.71	O.
15	45.37	24.40	.51	20.97	20.46	O.
16	46.00	32.52	1.22	13.48	12.26	O.
17	45.62	32.81	1.01	12.81	11.80	O.
18	45.60	32.82	.93	12.78	11.85	O.
Average for periods 15-18...	—	—	—	16.41	15.64	

DISCUSSION.

It is apparent from the tables that only a small percentage of the phosphorus of the basal ration was absorbed and retained in the body of the animal. Figures for the after period confirm this observation in the pre-period and show incidentally that the feeding of bonemeal had no lasting effect upon the absorption of phosphorus from the basal ration after the feeding of bonemeal had been stopped.

In order to calculate the percentage absorption of phosphorus from the phosphatic supplement during the experimental period, there seems no alternative but to assume that the absorption of phosphorus from the basal ration remained approximately constant throughout the experiment. The percentage absorption of phosphorus from the basal ration is calculated from the averages for the pre- and post-experimental periods. Furthermore, it must be mentioned that the phosphorus in the food minus that present in the faeces (i.e. the P which passed through the gut plus that excreted through the wall of the large intestine) is regarded as the phosphorus absorbed in these investigations. This interpretation is admittedly not quite correct, but appears to be the simplest way of providing comparative results.

The averages for any one period are given of the analyses made after the animal had adapted itself to the treatment of that period. For instance, in Table 2 the absorption for periods 5 and 6 are much too high, as naturally the bonemeal fed had not yet had time to pass through the animal's gut.

Calculated on the basis mentioned above, the percentage absorption of P_2O_5 of B.B. bonemeal given is 77 per cent. for bovine No. 2696 and 67 per cent. for bovine No. 3654.

Tables 3 and 4 show great difference in the absorption of CaO from day to day.

EXPERIMENT 2.

THE AVAILABILITY OF PHOSPHORUS IN SODIUM PHOSPHATE.

Duration of Experiment: 25.11.30-17.12.30.

The quantities of feed in the basal ration were changed slightly as the animals began to leave some of the hay. Bovine 2696 was therefore given 3.5 Kg. fanko and 3 Kg. hay. Bovine 3654 was given 3 Kg. fanko and 2.5 Kg. hay during the interval of a fortnight and for the experiment to be reported.

The samples of urine and faeces for analysis were taken for 48-hour periods of collection to reduce the number of analyses without affecting the reliability of the results.

The results of the analyses are given in the following tables:—

TABLE 5.—EXPERIMENT 2.

 P_2O_5 BALANCE OF BOVINE 2696 :

48-hour Period.	Intake P_2O_5 gm.	Outgo P_2O_5 gm.		Absorption P_2O_5 gm.	Retention P_2O_5 gm.	Phosphate Supplement.
		Faeces.	Urine.			
1	47.58	26.25	.29	21.33	21.04	O.
2	41.73	26.90	.30	14.83	14.53	O.
3	42.78	33.95	.20	8.83	8.63	O.
Average...	—	—	—	15.00	14.73	
4	105.28	29.70	.43	75.58	75.15	62.5 gm. P_2O_5 as sodium phosphate.
5	105.28	32.30	.34	72.98	72.64	" "
6	105.28	30.40	.76	74.88	74.12	" "
7	105.28	36.10	1.34	69.18	67.84	" "
Average for periods 6 and 7	—	—	—	72.03	70.98	
8	42.78	31.40	.39	11.38	10.99	O.
9	42.78	31.80	.38	10.98	10.60	O.
10	42.78	29.30	.46	13.48	13.02	O.
11	42.78	29.0	.23	13.78	13.55	O.
Average for periods 10 and 11	—	—	—	13.63	13.29	

TABLE 6.—EXPERIMENT 2.

 P_2O_5 BALANCE OF BOVINE 3354 :

48-hour Period.	Intake P_2O_5 gm.	Outgo P_2O_5 gm.		Absorption P_2O_5 gm.	Retention P_2O_5 gm.	Phosphate Supplement.
		Faeces.	Urine.			
1	38.40	18.10	.19	20.30	20.11	O.
2	39.23	22.70	.17	16.53	16.36	O.
3	40.02	25.50	.16	14.52	14.36	O.
Average...	—	—	—	17.12	16.94	
4	103.13	31.50	.14	71.63	71.49	62.5 gm. P_2O_5 as sodium phosphate.
5	103.12	26.60	.20	76.52	76.32	" "
6	102.87	25.23	.24	77.64	77.40	" "
7	102.58	24.90	.15	77.68	77.53	" "
Average for periods 6 and 7	—	—	—	77.66	77.47	
8	39.73	19.30	.19	20.43	20.24	O.
9	40.08	24.90	.19	15.18	14.99	O.
10	39.91	25.30	.22	14.61	14.39	O.
11	40.05	21.80	.17	18.25	18.08	O.
Average for periods 10 and 11	—	—	—	16.43	16.24	

TABLE 7.—EXPERIMENT 2.

CaO BALANCE OF BOVINE 2696 :

48-hour Period.	Intake CaO gm.	Outgo CaO gm.		Absorption CaO gm.	Retention CaO gm.	Phosphate Supplement.
		Faeces.	Urine.			
1	93.62	64.9	4.67	28.72	24.05	O.
2	96.46	120.6	5.06	-24.14	-29.20	O.
3	99.41	98.0	2.55	1.41	-1.14	O.
Average . .	—	—	—	2.00	-2.10	
4	99.41	49.7	4.77	49.71	44.94	62.5 gm. P ₂ O ₅ as sodium phosphate.
5	99.34	31.75	1.68	67.59	65.91	" "
6	99.82	28.8	1.80	71.02	69.22	" "
7	99.00	37.0	1.52	62.00	60.48	" "
Average for periods 6 and 7	—	—	—	66.51	64.85	
8	98.87	46.4	1.60	52.47	50.87	O.
9	99.00	53.7	3.10	45.30	42.20	O.
10	99.21	60.8	4.11	38.41	34.30	O.
11	99.14	59.0	2.59	40.14	37.55	O.
Average for periods 10 and 11	—	—	—	39.28	35.93	

TABLE 8.—EXPERIMENT 2.

CaO BALANCE OF BOVINE 3654.

48-hour Period.	Intake CaO gm.	Outgo CaO gm.		Absorption CaO gm.	Retention CaO gm.	Phosphate Supplement.
		Faeces.	Urine.			
1	90.32	73.00	4.50	17.32	12.82	O.
2	91.65	91.10	6.19	.55	-5.64	O.
3	94.55	100.00	6.72	-5.45	-12.17	O.
Average . .	—	—	—	4.14	-1.67	
4	96.50	55.20	2.86	41.30	38.44	62.5 gm. P ₂ O ₅ as sodium phosphate.
5	96.18	24.35	2.50	71.83	69.33	" "
6	95.51	24.60	1.38	70.91	39.53	" "
7	94.51	24.90	.93	69.61	68.68	" "
Average for periods 6 and 7	—	—	—	70.26	69.11	O.
8	93.50	25.81	1.36	67.69	66.33	O.
9	94.57	71.30	2.54	23.27	20.73	O.
10	93.70	69.50	2.42	24.20	21.78	O.
11	94.35	60.35	3.82	34.00	30.18	O.
Average for periods 10 and 11	—	—	—	29.10	25.98	

DISCUSSION.

The absorption of phosphorus from the basal ration in the case of Bovine 2696 corresponds well with that in Experiment 1. Bovine No. 3654, however, shows an increased absorption of P_2O_5 .

In calculating the percentage absorption of phosphorus from sodium phosphate on the same basis as in Experiment 1, Bovine No. 2696 shows 93 per cent, and Bovine No. 3654 shows 97 per cent.

The calcium content of the ration was again kept constant by adding 70 gm. calcium carbonate to the basal ration for the entire period. A remarkable observation was made with regard to calcium absorption, viz., that during the period which sodium phosphate was given the absorption of calcium was vastly increased. Furthermore, the elimination of calcium through the kidneys also shows a distinct decrease during the period of phosphate feeding. Apparently, an increase in phosphorus given as sodium phosphate favoured the absorption and utilization of Ca by the animal. Hence the influence of varying $CaO:P_2O_5$ ratios in the diet upon the absorption of Ca and P was dealt with at the present stage of the experiment. The results obtained will, therefore, be presented and discussed here before continuing the work upon the assimilation of phosphorus in phosphatic products.

There is no doubt, however, that in these experiments the phosphorus of disodium phosphate was more available to the bovines used than that of B.B. bouemeal.

EXPERIMENT 3.

THE ABSORPTION OF P AND Ca FROM A DIET CONTAINING
DIFFERENT RATIOS OF Ca AND P.

Duration of Experiment: 30.1.31-31.7.31.

The bovines were considered separately as the one was not used as a direct duplicate of the other.

A. The ration of Bovine 2696 consisted of:—

- 3 kg. fanko;
- 2.5 kg. yellow crushed maize;
- 3.4 kg. hay;
- 25 gm. casein;
- 45 gm. sodium chloride;
- water *ad lib.*

It was realized that 8-day periods were rather short and that it would be difficult to obtain reliable figures for a period free from effects of the ratio of the previous period.

Hence each experimental period was extended.

Malan and Du Toit (1932) regard 1.5 oz. of disodium phosphate as the required daily dose for bovines for optimum growth under ranching conditions.

It was, therefore, decided to keep the Ca intake of bovine 2696 high and to decrease the intake of sodium phosphate from excess to 45 gm. daily. Then by varying the calcium content of the diet an attempt was made to determine the point of best absorption of P.

TABLE 9.—EXPERIMENT 3.

 P_2O_5 BALANCE OF BOVINE 2696.

48-hour Period.	Intake P_2O_5 gm.	Outgo P_2O_5 gm.		Absorp- tion P_2O_5 gm.	Reten- tion P_2O_5 gm.	Supplement.	
		Faeces.	Urine.			CaO.	P_2O_5 .
1.....	48.57	43.30	.39	5.27	4.88	78.4 gm. CaO as CaCO ₃	—
2.....	49.98	40.61	.31	9.37	9.06	"	—
3.....	52.14	46.23	.32	5.91	5.59	"	—
4.....	52.14	47.93	.31	4.21	3.90	"	—
Average....	—	—	—	6.19	5.86		
5.....	105.61	43.98	.33	61.63	61.30	78.4 gm. CaO as CaCO ₃	57 gm. P_2O_5 as Na ₂ HPO ₄ + 12 H ₂ O.
6.....	109.53	64.17	.57	45.36	44.79	"	"
7.....	107.23	44.24	1.24	62.99	61.75	"	"
8.....	106.67	51.16	2.17	55.51	53.34	"	"
9.....	106.78	55.44	1.62	51.34	49.72	"	"
10.....	106.95	67.13	2.80	39.82	37.02	"	"
11.....	106.78	65.34	1.80	41.44	39.64	"	"
Average for period 7-11	—	—	—	59.22	48.29		
12.....	88.57	54.32	.88	34.25	33.37	78.4 gm. CaO as CaCO ₃	38.88 gm. P_2O_5 as Na ₂ HPO ₄ + 12 H ₂ O.
13.....	88.74	48.55	.67	40.19	39.52	"	"
14.....	88.82	53.73	.92	35.09	34.17	"	"
15.....	75.95	42.24	.76	33.71	32.95	78.4 gm. CaO as CaCO ₃	25.92 gm. P_2O_5 as Na ₂ HPO ₄ + 12 H ₂ O.
16.....	75.78	43.87	.90	31.81	30.91	"	"
17.....	75.78	39.71	1.04	36.07	36.03	"	"
18.....	75.78	48.58	.70	27.20	26.50	"	"
19.....	75.92	44.13	1.25	31.79	30.54	"	"
20.....	75.40	55.47	1.25	19.93	18.68	"	"
21.....	75.89	54.87	1.33	21.02	19.69	"	"
22.....	76.00	55.40	.89	20.60	19.71	"	"
23.....	75.78	47.39	.68	27.39	26.71	"	"
24.....	75.84	57.00	.41	18.84	18.43	"	"
25.....	75.78	53.50	1.00	22.28	21.28	"	"
26.....	75.78	47.33	.62	28.45	27.83	"	"
27.....	75.61	47.88	.60	27.73	27.13	"	"
28.....	73.59	50.13	.68	23.46	22.78	"	"
29.....	73.69	47.96	.61	25.73	25.12	"	"
30.....	73.59	47.00	.49	26.59	26.10	"	"
31.....	73.67	52.24	.55	21.43	20.88	"	"
32.....	67.23	47.54	.48	19.69	19.21	"	"
Average for periods 16-32	—	—	—	25.29	24.50		
33.....	67.14	45.55	.59	21.59	21.00	78.4 gm. CaO as CaCO ₃	19.44 gm. P_2O_5 as Na ₂ HPO ₄ + 12 H ₂ O.
34.....	67.01	42.23	.39	24.78	24.39	"	"
35.....	69.07	48.72	3.47	20.35	16.88	"	"
36.....	68.96	48.36	6.80	20.60	13.80	"	"
37.....	69.07	47.00	2.75	22.07	19.32	"	"
38.....	69.23	39.28	2.36	29.95	27.59	"	"
39.....	68.96	42.04	.81	26.92	26.11	"	"
40.....	69.26	46.10	1.24	23.16	21.92	"	"
41.....	69.07	38.45	1.04	30.62	29.58	"	"
42.....	69.34	38.45	1.09	30.89	29.80	"	"
43.....	68.27	44.33	1.48	23.94	22.46	"	"
44.....	68.37	45.94	1.14	22.43	21.29	"	"
45.....	68.54	44.86	.64	23.68	23.04	"	"

PHOSPHORUS, CALCIUM AND PROTEIN METABOLISM.

TABLE 9.—EXPERIMENT 3—(continued).

48-hour Period.	Intake P ₂ O ₅ gm.	Outgo P ₂ O ₅ gm.		Absorp- tion P ₂ O ₅ gm.	Reten- tion P ₂ O ₅ gm.	Supplement.	
		Faeces.	Urine.			CaO.	P ₂ O ₅ .
46.....	68.43	51.44	.62	16.99	16.37	78.4 gm. CaO as CaCO ₃	19.44 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
47.....	68.54	47.86	.53	20.68	20.15
48.....	68.80	41.62	.73	27.18	26.45
Average for periods 34-48	—	—	—	24.28	22.61		
49.....	68.4	50.38	1.08	18.0	16.9	56 gm. CaO as CaCO ₃	19.44 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
50.....	68.5	39.65	3.36	28.2	24.8
51.....	68.8	41.14	4.45	27.7	23.2
52.....	76.6	46.18	1.07	30.4	29.3
53.....	76.6	48.54	.90	28.1	27.2
54.....	76.2	45.00	.44	31.2	30.8
55.....	76.3	40.37	.38	35.9	35.5
56.....	76.1	40.99	.66	35.1	34.4
57.....	75.7	41.70	.76	34.0	33.2
58.....	75.9	40.57	.56	35.3	34.7
Average for periods 51-58	—	—	—	32.2	31.0		
59.....	75.5	46.5	.76	29.0	28.2	28 gm. CaO as CaCO ₃	19.44 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
60.....	75.7	49.8	.75	25.9	25.1
61.....	75.9	51.9	.53	24.0	23.5
62.....	71.5	53.2	.45	18.3	17.8
63.....	71.3	50.0	.50	21.3	20.8
64.....	71.3	55.0	.46	16.3	15.8
65.....	71.3	52.0	.41	19.3	18.9
66.....	71.3	58.6	.52	12.7	12.2
67.....	71.3	60.7	.42	10.6	10.2
68.....	71.3	47.1	.46	24.2	23.7
Average for periods 61-68	—	—	—	18.3	17.9		
69.....	71.3	55.6	.55	15.7	15.1	O	19.44 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
70.....	71.3	58.4	1.09	12.9	11.8	O	..
71.....	71.3	52.4	2.72	18.9	16.2	O	..
72.....	71.5	44.8	3.15	26.7	23.5	O	..
73.....	71.0	52.1	4.07	18.9	14.8	O	..
74.....	70.7	60.4	4.60	10.3	5.7	O	..
75.....	70.7	53.8	2.45	16.9	14.4	O	..
76.....	70.7	51.8	1.28	18.9	17.6	O	..
77.....	70.8	46.5	.85	24.3	23.4	O	..
78.....	70.7	49.0	1.09	21.7	20.6	O	..
Average for periods 71-78	—	—	—	19.6	17.0		
79.....	73.3	38.0	.66	35.3	34.6	56 gm. CaO as CaCO ₃	19.44 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
80.....	73.3	38.7	.52	34.6	34.1
81.....	73.3	39.0	.72	34.3	33.6
82.....	73.5	39.3	.41	34.2	33.8
83.....	73.4	38.7	.74	34.7	34.0
84.....	73.6	43.2	.64	30.4	29.8
85.....	73.6	44.1	.57	29.5	28.9
86.....	73.6	44.3	.62	29.3	28.7
87.....	73.3	39.9	.89	33.4	32.5
88.....	73.5	43.6	.70	29.9	29.2
Average for periods 81-88	—	—	—	32.0	31.3		

TABLE 10.—EXPERIMENT 3.

CaO BALANCE OF BOVINE 2696.

48-hour Period.	Intake CaO gm.	Output CaO gm.		Absorp- tion CaO gm.	Reten- tion CaO gm.	Supplement.	
		Faeces.	Urine.			CaO.	P ₂ O ₅ .
1.....	113.59	87.60	5.08	25.99	20.91	78.4 gm. CaO as CaCO ₃	0.
2.....	118.88	89.20	5.84	29.68	24.84	..	0.
3.....	118.46	82.00	5.38	36.46	31.08	..	0.
4.....	118.53	90.80	6.95	27.73	20.78	..	0.
Average....	—	—	5.81	29.97	24.40
5.....	110.98	71.30	4.61	39.68	35.07	78.4 gm. CaO as CaCO ₃	57 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
6.....	121.46	82.8	3.67	38.66	34.99
7.....	122.81	82.6	1.97	40.21	38.24
8.....	121.58	77.52	1.68	44.06	42.38
9.....	121.75	87.20	1.68	34.55	32.87
10.....	122.37	84.4	2.35	37.97	35.62
11.....	122.22	84.45	1.44	37.77	36.33
Average for periods	—	—	—	38.91	37.09
7-11.....	—	—	—	38.91	37.09
12.....	119.25	73.50	2.55	45.75	43.20	78.4 gm. CaO as CaCO ₃	38.88 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
13.....	120.32	72.52	1.20	47.80	46.60
14.....	119.20	79.10	.82	40.10	39.28
Average for periods	—	—	—	43.95	42.94
13 and 14	—	—	—	43.95	42.94
15.....	120.21	70.73	.95	49.48	48.53	78.4 gm. CaO as CaCO ₃	25.92 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
16.....	119.23	74.50	1.71	44.73	43.02
17.....	119.95	67.70	1.76	52.25	50.49
18.....	119.50	86.30	1.67	33.20	31.53
19.....	119.44	86.30	1.37	33.14	31.77
20.....	119.25	76.00	2.17	43.25	41.08
21.....	120.32	83.20	1.37	37.12	35.75
22.....	120.52	90.80	1.73	29.72	27.99
23.....	119.78	82.80	2.35	36.98	34.63
24.....	120.72	71.90	2.70	48.82	46.12
25.....	120.05	91.90	2.77	28.15	25.38
26.....	124.03	74.50	2.40	49.53	47.13
27.....	123.67	75.00	2.66	48.67	46.01
28.....	124.30	73.40	3.65	50.90	47.25
29.....	124.54	70.0	2.67	54.54	51.87
30.....	121.03	69.80	3.15	54.23	51.08
31.....	124.61	84.06	2.70	40.55	37.85
32.....	124.19	76.76	2.30	47.43	45.13
Average for periods	—	—	—	43.13	40.82
16-32....	—	—	—	43.13	40.82
33.....	123.69	89.92	2.18	33.77	31.59	78.4 gm. CaO as CaCO ₃	19.44 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
34.....	123.80	77.02	1.88	46.78	44.90

PHOSPHORUS, CALCIUM AND PROTEIN METABOLISM.

TABLE 10.—EXPERIMENT 3—(continued).

48-hour Period.	Intake CaO gm.	Outgo CaO gm.		Absorp- tion CaO gm.	Reten- tion CaO gm.	Supplement.	
		Faeces.	Urine.			CaO.	P ₂ O ₅ .
35.....	123·81	90·68	1·52	33·13	31·61	78·4 gm. CaO as CaCO ₃	19·44 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
36.....	123·15	101·90	1·40	21·25	19·85	"	"
37.....	123·86	107·71	1·76	16·15	14·39	"	"
38.....	124·03	77·48	2·67	46·55	43·88	"	"
39.....	123·15	70·28	2·58	52·87	50·29	"	"
40.....	123·51	83·62	1·78	39·89	38·11	"	"
41.....	122·17	79·12	1·43	43·05	41·62	"	"
42.....	122·90	87·72	1·25	35·18	33·93	"	"
43.....	123·17	87·90	1·91	35·27	33·36	"	"
44.....	120·14	80·68	1·49	39·46	37·97	"	"
45.....	120·33	78·23	2·71	42·10	39·39	"	"
46.....	121·06	89·62	1·93	31·44	29·51	"	"
47.....	121·87	86·83	1·92	35·04	33·12	"	"
48.....	121·15	82·53	2·00	38·62	36·62	"	"
Average for periods 34-48....	—	—	—	37·12	35·23		
49.....	97·9	102·5	1·9	— 4·6	— 6·5	56 gm. CaO as CaCO ₃	19·44 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
50.....	97·8	91·7	1·4	6·1	4·7	"	"
51.....	98·6	75·0	1·3	23·6	22·3	"	"
52.....	98·4	72·0	1·9	26·4	24·5	"	"
53.....	98·6	77·2	2·6	21·4	18·8	"	"
54.....	99·3	69·0	1·0	30·3	29·3	"	"
55.....	99·3	62·3	1·7	37·0	35·3	"	"
56.....	97·5	64·6	1·7	32·9	31·2	"	"
57.....	96·9	66·0	1·4	30·9	29·5	"	"
58.....	97·1	72·9	1·5	24·2	22·7	"	"
Average for periods 51-58....	—	—	—	28·3	26·7		
59.....	68·9	74·1	3·0	— 5·2	— 8·2	28 gm. CaO as CaCO ₃	19·44 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
60.....	68·0	67·8	2·9	·2	— 2·7	"	"
61.....	68·6	58·6	1·3	10·0	8·7	"	"
62.....	72·4	57·0	1·7	15·4	13·7	"	"
63.....	72·2	58·7	2·3	13·5	11·2	"	"
64.....	71·6	58·7	2·3	12·9	10·6	"	"
65.....	72·0	50·6	2·6	21·4	18·8	"	"
66.....	71·7	50·0	2·5	21·7	19·2	"	"
67.....	71·4	56·6	2·0	14·8	12·8	"	"
68.....	72·0	55·9	1·5	16·1	14·6	"	"
Average for periods 61-68....	—	—	—	15·7	13·7		
69.....	43·6	47·5	1·9	— 3·9	— 5·8	0.	19·44 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
70.....	43·2	47·5	1·3	— 4·3	— 5·6	0.	"
71.....	43·8	49·8	1·2	— 6·0	— 7·2	0.	"

TABLE 10.—EXPERIMENT 3—(continued).

48-hour Period.	Intake CaO gm.	Outgo CaO gm.		Absorp- tion CaO gm.	Reten- tion CaO gm.	Supplement.	
		Faeces.	Urine.			CaO.	P ₂ O ₅ .
72.....	44.4	39.2	1.3	5.2	3.9	O.	19.44 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
73.....	43.9	49.1	1.5	— 5.2	— 6.7	O.	"
74.....	36.3	40.1	1.8	— 3.8	— 5.6	O.	"
75.....	35.4	28.7	2.8	6.7	3.9	O.	"
76.....	35.7	35.0	1.4	.7	— .7	O.	"
77.....	36.2	29.5	1.5	6.7	5.2	O.	"
78.....	36.0	31.6	1.6	4.4	2.8	O.	"
Average for periods 71-78....	—	—	—	1.1	— .6		
79.....	93.7	64.2	1.9	29.5	27.6	56 gm. CaO as CaCO ₃	19.44 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
80.....	93.9	67.0	2.0	26.9	24.9	"	"
81.....	93.5	65.2	1.4	28.3	26.9	"	"
82.....	93.7	60.3	1.8	33.4	31.6	"	"
83.....	92.9	56.8	1.4	36.1	34.7	"	"
84.....	93.9	67.5	2.2	26.4	24.2	"	"
85.....	93.3	60.4	1.2	32.9	31.7	"	"
86.....	94.0	60.4	3.0	33.6	30.6	"	"
87.....	92.7	56.6	2.2	36.1	33.9	"	"
88.....	93.0	71.8	1.2	21.2	20.0	"	"
Average for periods 81-88....	—	—	—	31.0	29.2		
Periods 79-88	is a repetition of periods			49-58.			

DISCUSSION.

At the outset it must be stated that the figures are very interesting for several periods but that conclusions and interpretations will be unwise at the present stage. This work will be followed by more work along the same lines to ascertain to what extent the absorption was influenced by factors other than the Ca:P ratio and which are left out of consideration in this publication. For the present, only salient points in the Ca and P metabolism of bovine 2696 kept under the conditions of the experiment will be mentioned.

(1) The animal was kept in the metabolism stable continuously for 176 days except for its walk to the weighbridge and paddock mentioned previously. No ill effects were noticed and the animal remained in excellent health and continued to show a good appetite throughout.

(2) Periods 49-58 in Table 9 showed the most favourable absorption of P, 32 gm. P_2O_5 or 43.5 per cent. being absorbed of a total intake of 73.5 gm. The ratio of CaO: P_2O_5 was 1:77.

It is interesting to note that the results given for periods 81-88 are almost identical with those for 49 to 58, as the P intake and the CaO: P_2O_5 ratio were made to coincide with that of the latter periods as an additional check.

(3) Periods 1-4, while the animal was on a high Ca and low P diet, show a high elimination of Ca via the kidneys when compared with any of the periods during which phosphorus was given.

This observation was a confirmation of that made in Experiments I and II. At the end of this series of investigations (see Table 16, periods 71-76), when the P_2O_5 content of the ration was decreased the Ca elimination through the kidneys rose to approximately the same figure recorded in periods 1-4.

(4) Very little CaO was absorbed from the ration when sufficient P but no Ca was present. In addition it may be added that very little P was absorbed at the beginning of the experiment, when high Ca and low P were present in the ration.

B. Bovine 3654 21.5.31.

Daily ration:—

- 2.5 kg. fanko;
- 2.5 kg. yellow crushed maize;
- 1.4 kg. hay;
- 20 gm. aleuronat;
- 35 gm. sodium chloride;
- 70 gm. calcium carbonate.

The animal received the ration given above for a period of three months prior to the beginning of the experiment. The hay was gradually reduced to as much as the animal could be induced to take. As in the case of bovine 2696 the initial ration was high in Ca and low in P. The calcium was then reduced in stages to zero ultimately, when the P_2O_5 in its turn was increased in one stage to 57 gm. P_2O_5 as sodium phosphate per 48-hour period and gradually decreased to what was found to be the best period of absorption in the case of bovine 2696, viz., 19.4 gm. P_2O_5 per period.

Finally bovine 3654 was placed on the ratio which gave optimum absorption in the case of bovine 2696, i.e. 56 gm. CaO and 19.4 gm. P_2O_5 , were added per 48-hour period to the ration as calcium carbonate and sodium phosphate respectively.

The results of the analyses are given in Tables 12 and 13.

TABLE 12.

P₂O₅ BALANCE OF BOVINE 3654.

48-hour Period.	Intake P ₂ O ₅ gm.	Outgo P ₂ O ₅ gm.		Absorp- tion P ₂ O ₅ gm.	Reten- tion P ₂ O ₅ gm.	Supplement.	
		Faeces.	Urine.			CaO.	P ₂ O ₅ .
1.....	38.1	30.0	.43	8.1	7.7	78.4 gm. CaO as CaCO ₃	O.
2.....	37.2	25.5	.28	11.7	11.4	"	O.
3.....	36.3	27.8	.21	8.5	8.3	"	O.
4.....	37.2	28.5	.24	8.7	8.5	"	O.
5.....	37.4	30.3	.25	7.1	6.8	"	O.
Average....	—	—	—	8.8	8.5		
6.....	36.2	27.0	.19	9.2	9.0	56 gm. ^{1/2} CaO as CaCO ₃	O.
7.....	36.5	26.9	.30	9.6	9.3	"	O.
8.....	37.8	27.3	.21	10.5	10.3	"	O.
9.....	37.8	26.8	.22	11.0	10.8	"	O.
10.....	37.7	27.4	.22	10.3	10.1	"	O.
11.....	37.7	24.4	.18	13.3	13.1	"	O.
12.....	37.1	26.5	.21	10.6	10.4	"	O.
13.....	37.5	28.3	.24	9.2	9.0	"	O.
14.....	37.1	28.0	.20	9.1	8.9	"	O.
15.....	38.1	31.1	.31	7.0	6.7	"	O.
Average for periods 8-15.....	—	—	—	10.1	9.9		
16.....	38.1	29.0	.25	9.1	8.8	28 gm. CaO as CaCO ₃	O.
17.....	38.8	25.8	.20	13.0	12.8	"	O.
18.....	39.0	27.2	.24	11.8	11.6	"	O.
19.....	39.2	27.1	.19	12.1	11.9	"	O.
20.....	39.0	25.5	.19	13.5	13.3	"	O.
21.....	39.0	24.2	.24	14.8	14.6	"	O.
22.....	38.9	24.9	.39	14.0	13.6	"	O.
23.....	39.1	24.2	.17	14.9	14.7	"	O.
24.....	38.7	22.4	.18	16.3	16.1	"	O.
25.....	39.1	23.1	.18	16.0	15.8	"	O.
Average for periods 18-25....	—	—	—	14.2	14.0		
26.....	39.1	24.7	.32	14.4	14.1	O.	O.
27.....	39.2	22.3	.47	16.9	16.4	O.	O.
28.....	38.8	22.1	.38	16.7	16.3	O.	O.
29.....	39.1	23.1	.40	16.0	15.6	O.	O.
30.....	39.0	24.6	.29	14.4	14.1	O.	O.
31.....	38.8	22.4	.41	16.4	16.0	O.	O.
32.....	39.2	20.2	.44	19.0	18.6	O.	O.
33.....	38.8	24.7	.28	14.1	13.8	O.	O.
34.....	39.2	21.9	.24	17.3	17.1	O.	O.
35.....	39.2	24.1	.29	15.1	14.8	O.	O.
Average for periods 28-35....	—	—	—	16.1	15.8		

TABLE 12—(continued).

48-hour Period.	Intake P_2O_5 gm.	Outgo P_2O_5 gm.		Absorp- tion P_2O_5 gm.	Reten- tion P_2O_5 gm.	Supplement.	
		Faeces.	Urine.			CaO.	P_2O_5 .
36.....	—	—	—	—	—	O.	57 gm. P_2O_5 as Na_2HPO_4 + 12 H_2O .
41.....	95.4	35.5	37.9	59.9	22.0	O.	"
42.....	95.6	30.0	30.3	65.6	35.3	O.	"
43.....	95.6	31.9	33.6	63.7	30.1	O.	"
Average....	—	—	—	63.1	29.1		
44.....	77.4	28.0	19.3	49.4	30.1	O.	38.88 gm. P_2O_5 as Na_2HPO_4 + 12 H_2O .
45.....	77.4	31.5	17.1	45.9	28.8	O.	"
46.....	77.3	27.4	15.1	49.9	34.8	O.	"
47.....	77.4	27.2	13.8	50.2	36.4	O.	"
48.....	77.6	29.2	12.9	48.4	35.5	O.	"
49.....	77.6	27.4	6.8	50.2	43.4	O.	"
50.....	77.6	30.7	9.9	46.9	37.0	O.	"
Average for periods							
46-50....	—	—	—	49.1	37.4		
51.....	58.8	28.5	7.9	30.3	22.4	O.	19.44 gm. P_2O_5 as Na_2HPO_4 + 12 H_2O .
52.....	58.9	28.7	8.6	30.2	21.6	O.	"
53.....	58.8	27.5	4.9	31.3	26.4	O.	"
54.....	58.7	24.3	3.3	34.4	31.1	O.	"
55.....	58.7	28.7	5.4	30.0	24.6	O.	"
56.....	60.1	25.7	4.2	34.4	30.2	O.	"
57.....	60.0	24.8	2.4	35.2	32.8	O.	"
58.....	59.9	27.4	2.4	32.5	30.1	O.	"
59.....	59.9	28.7	6.4	31.2	24.8	O.	"
60.....	59.9	23.2	5.7	36.7	31.0	O.	"
Average for periods							
53-60....	—	—	—	33.2	28.9		
61.....	59.9	26.3	6.2	33.6	27.4	56 gm. CaO as $CaCO_3$	19.44 gm. P_2O_5 as Na_2HPO_4 + 12 H_2O .
62.....	59.9	21.8	1.7	38.1	36.4	"	"
63.....	59.9	23.6	.6	36.3	35.7	"	"
64.....	59.9	23.8	2.0	36.1	34.1	"	"
65.....	59.7	24.2	1.3	35.5	34.2	"	"
66.....	59.7	25.4	2.6	34.3	31.7	"	"
67.....	59.9	23.5	2.4	36.4	34.0	"	"
68.....	59.9	26.6	2.6	33.3	30.7	"	"
69.....	59.9	21.1	2.2	38.8	36.6	"	"
70.....	59.8	24.7	3.7	35.1	31.4	"	"
Average for periods							
63-70....	—	—	—	35.7	33.6		

TABLE 13.
CaO BALANCE OF BOVINE 3654.

48-hour Period.	Intake CaO gm.	Outgo CaO gm.		Absorp- tion CaO gm.	Reten- tion CaO gm.	Supplement.	
		Faeces.	Urine.			CaO.	P ₂ O ₅ .
1.....	99.7	69.4	9.3	30.3	21.0	78.4 gm. CaO as CaCO ₃	O.
2.....	97.5	68.0	6.5	29.5	23.0	"	O.
3.....	95.4	60.4	4.4	35.0	30.6	"	O.
4.....	96.8	61.4	4.9	35.4	30.5	"	O.
5.....	98.0	67.8	6.7	30.2	23.5	"	O.
Average....	97.5	—	6.4	32.1	25.7		
6.....	72.3	54.6	4.9	17.7	12.8	56 gm. CaO as CaCO ₃	O.
7.....	72.7	55.8	8.0	16.9	8.9	"	O.
8.....	75.7	51.2	4.9	24.5	19.6	"	O.
9.....	76.2	55.0	4.7	21.2	16.5	"	O.
10.....	75.4	47.4	5.3	28.0	22.7	"	O.
11.....	74.9	41.6	3.7	33.3	29.6	"	O.
12.....	74.7	44.7	4.4	30.0	25.6	"	O.
13.....	75.0	46.2	3.4	38.8	35.4	"	O.
14.....	74.6	50.2	3.3	24.4	21.1	"	O.
15.....	77.0	55.4	3.3	21.6	18.3	"	O.
Average for periods 8-15	—	—	—	27.7	23.6		
16.....	49.1	44.2	2.1	4.9	2.8	28 gm. CaO as CaCO ₃	O.
17.....	49.2	30.6	3.8	18.6	14.8	"	O.
18.....	48.4	34.2	4.9	14.2	9.3	"	O.
19.....	49.4	33.9	3.5	15.5	12.0	"	O.
20.....	49.1	31.3	1.9	17.8	15.9	"	O.
21.....	49.2	32.7	2.6	16.5	13.9	"	O.
22.....	48.5	33.0	3.2	18.5	15.3	"	O.
23.....	48.9	33.5	3.4	15.4	12.0	"	O.
24.....	48.6	29.2	1.7	19.4	17.7	"	O.
25.....	48.8	29.3	2.7	19.5	16.8	"	O.
Average for periods 18-25	—	—	—	17.1	14.1		
26.....	20.8	29.4	1.3	8.6	9.9	O.	O.
27.....	21.1	19.2	1.7	1.9	.2	"	O.
28.....	20.3	17.2	3.0	3.1	.1	"	O.
29.....	20.9	14.2	2.7	6.7	4.0	"	O.
30.....	20.6	16.5	1.2	4.1	2.9	"	O.
31.....	20.5	16.0	2.1	4.5	2.4	"	O.
32.....	21.2	13.5	2.4	7.7	5.3	"	O.
33.....	20.1	16.8	2.0	3.3	1.3	"	O.
34.....	20.9	15.6	2.5	5.3	2.8	"	O.
35.....	20.7	15.8	1.5	4.9	3.4	"	O.
Average for periods 28-35	—	—	—	5.0	2.8		
36.....	—	—	—	—	—	O.	57 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
41.....	17.1	13.1	1.1	4.0	2.9	O.	"
42.....	17.8	11.6	.6	6.2	5.6	O.	"
43.....	17.5	13.3	.9	4.2	3.3	O.	"
Average....	—	—	—	4.8	3.9		

TABLE 13—(continued).

48-hour Period.	Intake CaO gm.	Outgo CaO gm.		Absorp- tion CaO gm.	Reten- tion CaO gm.	Supplement.	
		Faeces.	Urine.			CaO.	P ₂ O ₅ .
44.....	17.5	12.2	.6	5.3	4.7	O.	38.88 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
45.....	17.7	13.3	.5	4.4	3.9	O.	..
46.....	17.9	12.4	.6	5.5	4.9	O.	..
47.....	17.7	12.6	.7	5.1	4.4	O.	..
48.....	17.6	13.5	.6	4.1	3.5	O.	..
49.....	18.5	10.8	.8	7.7	6.9	O.	..
50.....	18.4	10.6	.8	7.8	7.0	O.	..
Average for periods	—	—	—	6.0	5.3		
46-50....	—	—	—	6.0	5.3		
51.....	17.9	9.7	1.0	8.2	7.2	O.	19.44 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
52.....	18.4	10.3	1.4	8.1	6.7	O.	..
53.....	18.3	10.6	1.1	7.7	6.6	O.	..
54.....	18.3	10.7	.8	7.6	6.8	O.	..
55.....	18.2	12.3	1.5	5.9	4.4	O.	..
56.....	17.6	8.7	1.1	8.9	7.8	O.	..
57.....	16.8	10.3	.5	6.5	6.0	O.	..
58.....	18.0	11.6	1.0	6.4	5.4	O.	..
59.....	18.1	12.7	1.5	5.4	3.9	O.	..
60.....	17.8	10.8	1.1	7.0	5.9	O.	..
Average for periods	—	—	—	6.9	5.9		
53-60....	—	—	—	6.9	5.9		
61.....	73.3	17.9	.7	55.4	54.7	56 gm. CaO as CaCO ₃	19.44 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
62.....	74.1	26.3	2.1	47.8	45.7
63.....	74.2	29.0	.6	45.2	44.6
64.....	73.8	38.4	.9	35.4	34.5
65.....	73.2	38.5	1.8	34.7	32.9
66.....	73.8	38.6	1.2	35.2	34.0
67.....	73.0	36.2	1.5	36.8	35.3
68.....	73.8	43.0	1.7	30.8	29.1
69.....	73.1	36.2	1.4	36.9	35.5
70.....	73.3	39.6	1.3	33.7	32.4
Average for periods	—	—	—	36.1	34.8		
63-70....	—	—	—	36.1	34.8		

DISCUSSION.

(1) The absorption of phosphorus gradually increased from 23.7 per cent. at the beginning of the experiment with the decrease in the supplement of CaCO₃ until a maximum of 41.3 per cent. was obtained, when no CaCO₃ was added. In other words, it appears that under the conditions of the experiment better absorption of phosphorus was recorded when both Ca and P were deficient in the diet, than when excess of Ca was given with a deficiency of P.

It is noteworthy that Theiler, Green and du Toit (1924) made an observation which bears out the above result. They noted that animals on a P deficient ration, receiving chalk showed an increased osteophagia over those receiving no chalk in their ration.

(2) The absorption of P was increased by giving a phosphatic supplement. However, it is interesting to note that for the highest supplement of P, i.e. periods 36-43, the greater portion of the absorbed P was eliminated through the kidneys, and as the P supplement decreased there was a decrease in the quantity of P in the urine. While 66 per cent. of the total P_2O_5 intake was absorbed only 30 per cent. was retained, the rest being eliminated via the kidneys. As the P supplement decreased the quantity of P_2O_5 absorbed decreased, but the portion retained remained approximately constant.

(3) During the final stage of the experiment 56 gm. CaO and 19.44 gm. P_2O_5 were again given per 48-hour period. As in the case of bovine 2696 the absorption of P from the basal ration was increased for this period. In other words, although only 19.44 gm. P_2O_5 were given as supplement (35.7-8.8) gm. P_2O_5 were absorbed, or (26.9-19.44) gm. must have been absorbed in addition to the 8.8 gm. of the pre-period.

(4) As indicated with Bovine 2696, calcium elimination via the kidneys is higher on a Ca high P low ration, than when the Ca supplement is decreased or when a phosphorus supplement is given.

A summary of the work done on the two bovines at different Ca:P ratios is given below, while comment thereupon is reserved until later, when such matters as the availability of P and Ca in the supplements, the proportion of proteins, carbohydrates, etc., of the ration, the amounts of supplements given, and other questions have been dealt with.

TABLE 11.

COMPARISON OF THE CaO : P_2O_5 RATIO WITH THE ABSORPTION IN TABLES 9 AND 10.

48-hour Period.	Intake.		CaO : P_2O_5 Ratio.	Absorption gm.		Retention gm.	
	CaO gm.	P_2O_5 gm.		CaO.	P_2O_5 .	CaO.	P_2O_5 .
1-4.....	117.4	50.7	1:0.43	30.0	6.2	24.4	5.9
7-11.....	122.0	107.3	1:0.88	38.9	50.2	37.1	48.3
16-32.....	121.4	74.8	1:0.62	43.1	25.3	40.8	24.5
34-48.....	122.5	68.7	1:0.56	37.1	24.3	35.2	22.6
51-58.....	98.2	75.2	1:0.77	28.3	32.2	26.7	31.0
61-68.....	71.5	72.0	1:1.0	15.7	18.3	13.7	17.9
71-78.....	39.0	70.9	1:1.82	1.1	19.6	— 0.6	17.0
81-88.....	93.4	73.5	1:0.79	31.0	32.0	29.2	31.3

TABLE 14.

COMPARISON OF THE $\text{CaO} : \text{P}_2\text{O}_5$ RATIO WITH THE ABSORPTION AND RETENTION IN TABLES 12 AND 13.

48-hour Period.	Intake.		$\text{CaO} : \text{P}_2\text{O}_5$ Ratio.	Absorption gm.		Retention gm.	
	CaO gm.	P_2O_5 gm.		CaO.	P_2O_5 .	CaO.	P_2O_5 .
1-5.....	97.5	37.2	1 : 0.38	32.1	8.8	25.7	8.5
8-15.....	75.4	37.6	1 : 0.50	27.7	10.1	23.6	9.9
18-25.....	48.9	39.0	1 : 0.80	17.1	14.2	14.1	14.0
63-70.....	73.5	59.8	1 : 0.81	36.1	35.7	34.7	33.6
28-35.....	20.6	39.0	1 : 1.89	5.0	16.1	2.8	15.8
53-60.....	17.9	59.5	1 : 3.32	6.9	33.2	5.9	28.9
46-50.....	18.0	77.5	1 : 4.31	6.0	49.1	5.3	37.4
41-43.....	17.5	95.5	1 : 5.46	4.8	63.1	3.9	29.1

EXPERIMENT 4.

INFLUENCE OF PHOSPHORUS UPON FOOD CONSUMPTION AND ABSORPTION.

Theiler, Green and du Toit (1924) raised the question whether cattle receiving bonemeal eat more food in putting on the extra weight or whether they utilize their food more economically. After carrying out some feeding trials they concluded that the animals receiving bonemeal consumed more hay than the control animals on the same ration, but without bonemeal. The other question of the utilization of the food had to be left until balance experiments could be carried out.

Orr (1929) considered minerals essential food constituents, and that the deficiency of one or more limited the utilization of the other food constituents. Woodman and Evans (1930) working with two fifteen months old wethers, concluded that "malnutrition on pasture of subnormal mineral content was directly due to the failure of the diet to supply the necessary inorganic materials for constructional purposes, and for maintaining the normal balance of minerals in the blood or tissues, and should not be ascribed even in part to an indirect effect, such as is embodied in the suggestion that mineral deficiency leads to under-nutrition of the animal, by causing a depression of its appetite and its capacity to digest the organic constituents of the herbage."

The work reported on in Experiment 4 was undertaken with a view to throw more light on the utilization of the food of bovines on rations containing more or less phosphorus and calcium respectively.

At this stage it may be mentioned that it was continually observed throughout the course of these investigations that both bovines ate the hay considerably better and more rapidly when phosphates were added to the ration. The phosphate did not affect the taste of the hay for the latter was given separately in all cases, while the phosphate was added to the fanko and maize.

The balances of proteins, carbohydrates, ether soluble extracts and fibre are given in the following tables.

TABLE 15.—EXPERIMENT 4.
NITROGEN BALANCE OF BOVINE 2696.

48-hour Period.	Intake N gm.	Outgo N gm.		Absorp- tion N gm.	Reten- tion N gm.	Supplement.	
		Faeces.	Urine.			CaO.	P ₂ O ₅ .
1.....	195.7	105.3	44.4	90.4	46.0	78.4 gm. CaO as CaCO ₃	O.
2.....	195.1	120.0	56.5	75.1	18.6	"	O.
3.....	201.4	119.7	34.8	81.7	46.9	"	O.
Average....	—	—	—	82.4	37.2		
4.....	201.4	109.5	68.3	91.9	23.6	78.4 gm. CaO as CaCO ₃	62.5 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
5.....	201.4	110.0	51.8	91.4	39.6	"	"
6.....	201.4	99.4	61.1	102.0	40.9	"	"
7.....	201.4	117.2	63.8	84.2	20.4	"	"
Average of periods 6 and 7..	—	—	—	93.1	30.7		
8.....	201.4	95.8	42.0	105.6	63.6	78.4 gm. CaO as CaCO ₃	O.
9.....	201.4	103.6	66.6	97.8	31.2	"	O.
10.....	201.4	107.5	57.0	93.9	36.9	"	O.
11.....	201.4	114.5	43.8	86.9	43.1	"	O.
Average of periods 10 and 11	—	—	—	90.4	40.0		

TABLE 16.—EXPERIMENT 4.
NITROGEN BALANCE OF BOVINE 3654.

48-hour Period.	Intake N gm.	Outgo N gm.		Absorp- tion N gm.	Reten- tion N gm.	Supplement.	
		Faeces.	Urine.			CaO.	P ₂ O ₅ .
1.....	166.0	85.0	27.0	81.0	54.0	78.4 gm. CaO as CaCO ₃	O.
2.....	161.0	88.0	30.8	73.0	42.2	"	O.
3.....	175.8	89.9	39.1	85.9	46.8	"	O.
Average....	—	—	—	80.0	47.7		
4.....	179.6	123.7	43.1	55.9	12.8	78.4 gm. CaO as CaCO ₃	62.5 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
5.....	179.5	88.3	47.5	91.2	43.7	"	"
6.....	177.8	89.0	39.5	88.8	49.3	"	"
7.....	176.0	96.0	26.1	80.0	53.9	"	"
Average of periods 6 and 7..	—	—	—	84.4	51.6		
8.....	174.0	68.0	32.0	106.0	74.0	78.4 gm. CaO as CaCO ₃	O.
9.....	176.0	81.0	37.9	95.0	57.1	"	O.
10.....	175.1	97.3	39.9	77.8	37.9	"	O.
11.....	176.0	78.5	36.2	97.5	61.3	"	O.
Average of periods 10 and 11	—	—	—	87.7	49.6		

TABLE 17.—EXPERIMENT 5.
NITROGEN BALANCE OF BOVINE 3654.

48-hour Period.	Nitro- gen Intake, gm.	Outgo N gm.		Absorp- tion N gm.	Reten- tion N gm.	Supplement.	
		Faeces.	Urine.			CaO.	P ₂ O ₅ .
1.....	174.28	82.84	64.20	91.44	27.24	78.4 gm. CaO as CaCO ₃	O.
2.....	171.46	67.79	47.41	103.67	56.26	"	O.
3.....	168.76	72.68	46.20	96.08	49.88	"	O.
4.....	171.36	76.18	57.50	95.18	37.68	"	O.
5.....	172.46	86.67	65.54	85.79	30.36	"	O.
Average....	—	—	—	—	40.3	—	—
10.....	172.76	86.70	64.30	86.06	21.76	56 gm. CaO as CaCO ₃	O.
11.....	172.86	73.95	51.20	98.91	47.71	"	O.
12.....	170.96	78.90	49.90	92.06	42.16	"	O.
13.....	172.16	77.38	55.35	94.78	39.43	"	O.
14.....	172.16	79.59	39.20	92.57	53.37	"	O.
15.....	173.96	80.91	52.65	93.05	40.40	"	O.
Average....	—	—	—	—	40.8	—	—
19.....	175.26	92.73	39.00	82.53	43.53	28 gm. CaO as CaCO ₃	O.
20.....	174.86	83.05	37.87	91.81	53.94	"	O.
21.....	174.86	77.40	46.40	97.46	51.06	"	O.
22.....	174.56	80.70	86.30	83.86	— 2.44	"	O.
23.....	175.06	80.70	48.06	84.36	36.30	"	O.
24.....	173.86	78.25	38.70	95.61	56.91	"	O.
25.....	175.06	74.18	53.32	100.88	47.56	"	O.
Average....	—	—	—	—	41.0	—	—
29.....	175.06	79.75	51.00	95.31	44.31	O.	O.
30.....	173.86	86.80	43.40	87.06	43.66	O.	O.
31.....	174.36	80.20	41.69	94.16	52.47	O.	O.
32.....	175.46	67.05	49.70	108.41	58.71	O.	O.
33.....	174.36	87.45	50.00	86.91	36.91	O.	O.
34.....	175.46	76.92	57.72	98.54	40.82	O.	O.
35.....	175.26	99.87	36.10	75.39	39.29	O.	O.
Average....	—	—	—	—	45.2	—	—
54.....	170.16	72.45	37.60	97.71	60.11	O.	19.9 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
55.....	170.26	85.60	82.75	84.66	1.91	O.	"
56.....	170.16	74.15	57.60	96.01	38.41	O.	"
57.....	169.96	72.87	52.99	97.09	44.10	O.	"
58.....	169.86	82.40	30.38	87.46	57.08	O.	"
59.....	169.86	85.60	66.90	84.26	17.36	O.	"
60.....	169.86	72.80	48.80	97.06	48.26	O.	"
Average....	—	—	—	—	38.2	—	—
64.....	167.85	68.60	52.15	99.25	47.10	56 gm. CaO as CaCO ₃	19.9 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
65.....	167.55	73.19	53.65	94.36	40.71	"	"
66.....	167.43	84.00	60.05	83.43	23.38	"	"
67.....	167.85	82.40	62.20	85.45	23.25	"	"
68.....	167.85	76.08	52.20	91.77	39.57	"	"
69.....	167.85	68.99	53.90	98.86	44.96	"	"
70.....	167.85	77.42	46.53	90.43	43.90	"	"
Average....	—	—	—	—	37.6	—	—

TABLE 18.—EXPERIMENT 4.
NITROGEN FREE EXTRACT BALANCE OF BOVINE 3654.

48-hour Period.	Intake Hay Kg.	Nitrogen free extract gm.			Supplement.	
		Intake.	Outgo.	Absorp- tion.	CaO.	P ₂ O ₅ .
1.....	2.63	8,466	2,111	6,355	78.4 gm. CaO as CaCO ₃	O.
2.....	2.31	8,329	1,560	6,769	"	O.
3.....	2.00	8,297	1,766	6,531	"	O.
4.....	2.30	8,325	1,811	6,514	"	O.
5.....	2.42	8,376	2,384	5,992	"	O.
Average.....	—	—	—	6,432		
10.....	2.46	8,393	1,870	6,523	56 gm. CaO as CaCO ₃	O.
11.....	2.47	8,398	1,796	6,602	"	O.
12.....	2.25	8,304	1,732	6,572	"	O.
13.....	2.39	8,363	1,694	6,669	"	O.
14.....	2.28	8,316	1,715	6,601	"	O.
15.....	2.60	8,453	1,984	6,469	"	O.
Average.....	—	—	—	6,573		
19.....	2.70	8,443	2,224	6,419	28 gm. CaO as CaCO ₃	O.
20.....	2.66	8,427	1,870	6,557	"	O.
21.....	2.65	8,423	1,911	6,512	"	O.
22.....	2.62	8,411	1,999	6,412	"	O.
23.....	2.68	8,435	1,884	6,551	"	O.
24.....	2.55	8,382	1,938	6,444	"	O.
25.....	2.68	8,435	1,831	6,604	"	O.
Average.....	—	—	—	6,500		
29.....	2.68	8,435	2,086	6,349	O.	O.
30.....	2.64	8,419	1,920	6,499	O.	O.
31.....	2.60	8,402	1,857	6,545	O.	O.
32.....	2.72	8,451	1,645	6,806	O.	O.
33.....	2.60	8,402	2,051	6,351	O.	O.
34.....	2.72	8,451	1,854	6,597	O.	O.
35.....	2.70	8,451	1,779	6,672	O.	O.
Average.....	—	—	—	6,546		
54.....	2.90	8,571	1,810	6,761	O.	19.9 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
55.....	2.92	8,579	2,036	6,543	O.	"
56.....	2.90	8,571	1,913	6,658	O.	"
57.....	2.87	8,558	1,835	6,723	O.	"
58.....	2.85	8,550	2,081	6,469	O.	"
59.....	2.85	8,550	2,290	6,260	O.	"
60.....	2.85	8,550	1,968	6,582	O.	"
Average.....	—	—	—	6,571		
64.....	2.85	8,552	1,851	6,701	56 gm. CaO as CaCO ₃	19.9 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
65.....	2.80	8,531	1,840	6,691	"	"
66.....	2.78	8,522	1,978	6,544	"	"
67.....	2.85	8,552	1,884	6,668	"	"
68.....	2.85	8,552	1,993	6,559	"	"
69.....	2.85	8,552	1,863	6,689	"	"
70.....	2.85	8,552	1,821	6,731	"	"
Average.....	—	—	—	6,655		

TABLE 19.
ETHER EXTRACT BALANCE OF BOVINE 3654.

48-hour Period.	Ether Extract gm.			Supplement.	
	Intake, gm.	Outgo, gm.	Absorp- tion.	CaO.	P ₂ O ₅ .
1.....	523.6	134.6	389.0	78.4 gm CaO as CaCO ₃	O.
2.....	515.9	102.0	413.9	"	O.
3.....	508.4	128.5	379.9	"	O.
4.....	515.7	120.9	394.8	"	O.
5.....	518.6	110.9	407.7	"	O.
Average.....	—	—	397.1		
10.....	519.5	116.6	402.9	56 gm. CaO as CaCO ₃	O.
11.....	519.8	103.0	416.8	"	O.
12.....	514.5	123.6	390.9	"	O.
13.....	517.8	107.5	410.3	"	O.
14.....	515.2	122.1	393.1	"	O.
15.....	522.9	134.3	388.6	"	O.
Average.....	—	—	400.4		
19.....	530.2	146.9	383.3	28 gm. CaO as CaCO ₃	O.
20.....	529.2	118.9	410.3	"	O.
21.....	528.9	137.4	391.5	"	O.
22.....	528.1	119.5	408.6	"	O.
23.....	529.7	144.7	385.0	"	O.
24.....	526.3	122.8	403.5	"	O.
25.....	529.7	118.0	411.7	"	O.
Average.....	—	—	399.1		
29.....	529.7	147.1	382.6	O.	O.
30.....	528.6	149.0	379.6	O.	O.
31.....	527.6	119.9	407.7	O.	O.
32.....	530.7	106.6	424.1	O.	O.
33.....	527.6	145.4	382.2	O.	O.
34.....	530.7	119.4	411.3	O.	O.
35.....	530.2	115.3	414.9	O.	O.
Average.....	—	—	400.3		
54.....	531.9	114.9	417.0	O.	19.9 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
55.....	532.4	119.2	413.2	O.	"
56.....	531.9	113.9	418.0	O.	"
57.....	531.2	111.0	420.2	O.	"
58.....	530.7	133.7	397.0	O.	"
59.....	530.7	117.3	403.4	O.	"
60.....	530.7	93.8	436.9	O.	"
Average.....	—	—	415.1		
64.....	537.2	120.7	416.5	56 gm. CaO as CaCO ₃	19.9 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
65.....	535.9	80.5	455.4	"	"
66.....	535.3	103.5	421.8	"	"
67.....	537.2	157.4	379.8	"	"
68.....	537.2	135.8	401.4	"	"
69.....	537.2	98.0	439.2	"	"
70.....	537.2	118.8	418.4	"	"
Average.....	—	—	418.9		

TABLE 20.
FIBRE BALANCE OF BOVINE 3654.

48-hour Period.	Fibre gm.			Supplement.	
	Intake.	Outgo.	Absorp- tion.	CaO.	P ₂ O ₅ .
1.....	1015·0	597·3	417·7	78·4 gm. CaO as CaCO ₃	O.
2.....	907·4	447·6	459·8	..	O.
3.....	803·1	546·3	256·8	..	O.
4.....	904·0	550·9	353·1	..	O.
5.....	944·3	730·6	203·7	..	O.
Average.....	—	—	338·2		
10.....	957·8	536·9	420·9	56 gm. CaO as CaCO ₃	O.
11.....	961·2	431·0	530·2	..	O.
12.....	887·2	455·9	431·3	..	O.
13.....	934·3	448·6	485·7	..	O.
14.....	897·3	531·4	365·9	..	O.
15.....	1004·9	513·5	491·4	..	O.
Average.....	—	—	454·2		
19.....	1031·2	626·1	405·1	28 gm. CaO as CaCO ₃	O.
20.....	1017·9	508·4	509·5	..	O.
21.....	1014·5	480·7	533·8	..	O.
22.....	1004·5	494·3	510·2	..	O.
23.....	1024·5	577·9	446·6	..	O.
24.....	981·2	469·9	511·3	..	O.
25.....	1024·5	516·8	507·7	..	O.
Average.....	—	—	489·2		
29.....	1024·5	475·1	549·4	O.	O.
30.....	1011·2	585·2	426·0	O.	O.
31.....	997·9	532·0	465·9	O.	O.
32.....	1037·9	470·0	567·9	O.	O.
33.....	997·9	608·4	389·5	O.	O.
34.....	1037·9	510·0	527·9	O.	O.
35.....	1031·2	498·4	532·8	O.	O.
Average.....	—	—	494·2		
54.....	1149·6	575·5	574·1	O.	19·9 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
55.....	1156·6	654·4	502·2	O.	..
56.....	1149·6	606·6	543·0	O.	..
57.....	1139·0	500·7	638·3	O.	..
58.....	1132·0	612·9	519·1	O.	..
59.....	1132·0	629·6	502·4	O.	..
60.....	1132·0	574·9	557·1	O.	..
Average.....	—	—	548·0		
64.....	1162·2	568·4	593·8	56 gm. CaO as CaCO ₃	19·9 gm. P ₂ O ₅ as Na ₂ HPO ₄ + 12 H ₂ O.
65.....	1144·1	571·2	572·9
66.....	1136·9	749·4	387·5
67.....	1162·2	693·9	468·3
68.....	1162·2	713·5	448·7
69.....	1162·2	585·5	576·7
70.....	1162·2	592·6	569·6
Average.....	—	—	516·8		

DISCUSSION.

(1) There was no significant difference in the absorption of any of the fractions determined during the periods given, i.e. when calcium and phosphorus supplements were given or omitted as stated in the tables.

(2) Only about 53 per cent. of the nitrogen given was absorbed while a high percentage of fibre was utilized by the animals.

(3) The absorption of ether extract during all these periods remained remarkably constant.

(4) It would appear that the Ca and P contents of the ration in the experiment under consideration did not affect the absorption of protein, carbohydrates, ether soluble extract, and fibre to any appreciable extent.

The work of du Toit *et al.* (1930, 1931, 1932) with sheep may be quoted as confirmative evidence, that food consumption increases with the addition of phosphatic supplements to rations, and that phosphorus deficiency is invariably associated with poor appetite.

The availability of phosphorus in precipitated calcium phosphate, degelatinized boneflour, ammonium phosphate, two brands of bonemeal and tricalcium phosphate, has been studied on two adult bovines. The results will be considered in conjunction with those being obtained for growing animals and published at a later stage.

SUMMARY.

(1) Results of balance experiments with two bovines on the availability of phosphorus in bonemeal and sodium phosphate are presented.

(2) The phosphorus in disodium phosphate was found to be practically 100 per cent. available, while that in bonemeal registered only about 70 per cent.

(3) The absorption of calcium and phosphorus at different levels of intake and with varying ratios in the diet of CaO and P₂O₅ has been studied and tables presented.

(4) It was found that the Ca eliminated through the kidneys increased when the animals were placed on a ration low in phosphorus.

(5) When phosphorus was added as sodium phosphate to a ration low in P the absorption and retention of Ca was influenced favourably.

(6) When the diet was high in calcium but low in phosphorus, the absorption of P was considerably less than when it was low in both.

(7) High phosphorus as sodium phosphate but low calcium in the diet resulted in a high absorption of P but in an extraordinary high elimination via the kidneys.

(8) For a daily supplement of 45 gm. of disodium phosphate, i.e. 9.72 gm. P_2O_5 , best absorption of P was obtained when 50 gm. calcium carbonate, i.e. 28 gm. CaO were added to the ration used.

(9) The utilization of protein, carbohydrates, ether soluble extracts and fibre has been studied under conditions of phosphorus and calcium deficiency and sufficiency, with apparently negative results.

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REFERENCES.

- DU TOIT, P. J., MALAN, A. I., ROSSOUW, S. D. (1930). Studies in Mineral Metabolism XII. Phosphorus in the sheep industry. *16th Rept. of the Dir. of Vet. Serv. and A.I.* Union of S.A. pp. 313-326.
- DU TOIT, P. J., MALAN, A. I., and GROENEWALD, J. W. (1931). Studies in Mineral Metabolism XVII. Phosphorus in the Nutrition of Sheep. *17th Rept. Dir. of Vet. Serv. and A.I.* Union of S.A., pp. 454-472.
- DU TOIT, P. J., MALAN, A. I., and GROENEWALD, J. W. (1932). This Report.
- DU TOIT, P. J. and GREEN, H. H. (1930). Dicalcium phosphate as a source of phosphorus in nutrition. *16th Rept. Dir. of Vet. Serv. and A.I.* Union of S.A., pp. 267-285.
- GREEN, H. H. (1928). Studies in mineral metabolism IV. Determination of phosphorus compounds in blood by dry combustion. *Jnl. Agric. Sc.*, Vol. 18, pp. 372-375.
- HENRICI, M. (1928). The phosphorus content of grasses of Bechnanaland in the course of their development. *13th and 14th Rept. Dir. Vet. Educ. and Res.*, pp. 1077-1208.
- HENRICI, M. (1930). The phosphorus content of grasses in the Eastern Transvaal. *16th Rept. Dir. Vet. Serv. and A.I.*, pp. 435-499.
- JURITZ, C. F. (1909). A study of the agricultural soils of the Cape Colony. Cape Times Limited, Capetown.
- MALAN, A. I., and DU TOIT, P. J. (1932). This Report.
- MALAN, A. I., GREEN, H. H., and DU TOIT, P. J. (1928). Studies in Mineral Metabolism V. Composition of Bovine blood on phosphorus deficient pasture. *Jnl. of Agric. Sc.*, Vol. 18, pp. 376-383.
- MALAN, A. I., and VAN DER LINGEN, G. W. B. (1931). Studies in mineral metabolism XVI. The micro-determination of some inorganic elements in blood and vegetation. *17th Rept. Dir. of Vet. Serv. and A.I.*, pp. 443-452.
- MEIGS, E. B., BLATHERWICK, N. R., and CARY, C.A. (1919). Further Contributions to the physiology of phosphorus and calcium metabolism of dairy cows. *Jnl. Biol. Chem.*, Vol. 40, pp. 409-500.
- ORR, J. B. (1929). Minerals in pastures. Publishers, H. K. Lewis & Co., London.
- THEILER, A., GREEN, H. H., and VILJOEN, P. R. (1915). A contribution to the study of deficiency disease, with special reference to the lamsiekte problem in South Africa. *3rd and 4th Rept. Dir. of Vet. Res.*, pp. 1-68.
- THEILER, A., VILJOEN, P. R., GREEN, H. H., DU TOIT, P. J., and MEIER, H. (1920). The cause and prevention of Lamsiekte. *Jnl. of Dept. of Agric.*, Vol. 1, pp. 221-244.

- THEILER, A., GREEN, H. H., DU TOIT, P. J. (1924). Phosphorus in the Livestock Industry. *Jnl. Dept. of Agric.*, Vol. 8, pp. 460-504.
- THEILER, A., VILJOEN, P. R., GREEN, H. H., DU TOIT, P. J., MEIER, H., and ROBINSON, E. M. (1927). Lamsiekte in Cattle in South Africa. *11th and 12th Rept. Dir. Vet. Educ. and Res.*, pp. 821-1361.
- VAN ZYL, J. P. (1928). Soil conditions in typical lamsiekte and styfsiekte soils, as revealed by pot cultures. *13th and 14th Rept. Dir. Vet. Educ. and Res.*, pp. 993-1036.
- WOOD, T. B. (1911). A course of practical work in agricultural chemistry for senior students. Cambridge: At the University Press.
- WOODMAN, H. E., and EVANS, R. E. (1930). The nutritive value of Pasture. *Jnl. Agric. Sc.*, Vol. 20, pp. 587-615.

Studies in Mineral Metabolism XXIII: Phosphorus and Iodine Supplements in Field Experiments with Sheep.

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INTRODUCTION.

Dr Torr, Malan and Rossouw (1930) have pointed out that, although a great deal of work has been done in South Africa on the problem of phosphorus deficiency in cattle, comparatively little is known about the rôle of phosphorus in the sheep industry. The overlooking of this problem in the smaller ruminant can be accounted for by the fact that the phosphorus deficiency in bovines is accompanied by serious diseases such as Lamsiekte or Styfsiekte, whereas in the sheep it is only when the deficiency becomes very acute that diseases directly concerned with a phosphorus deficiency may make their appearance. Farmers are, nevertheless, extensively feeding bonemeal and other phosphorus-containing licks to their sheep in different parts of the Union, and it is generally believed that these supplements are having beneficial results. As no controlled investigations have so far been made in the field, it is impossible to state with any certainty, whether these contentions are true, or to what extent phosphorus supplements improve the condition of sheep. To obtain more conclusive and accurate information, experimental work was undertaken at Armoedsvlakte in Vryburg District, and the data accumulated, although limited in some respects, are of considerable interest. It was considered that the observations would also yield some information about the pastoral conditions of Bechuanaland and whether it would be possible to make a success of sheep farming, especially Merino breeding in that locality. Up to the present farmers there have not had any success in keeping better bred woolled sheep. The feeding of bonemeal to cattle has so improved the productivity of this animal in Bechuanaland, and it could be considered that a big difference in the condition, etc., of sheep would be achieved by correcting the phosphorus deficiency with bonemeal.

EXPERIMENTAL WORK.

EXPERIMENTAL ANIMALS.

As was reported by Bekker and Rossouw (1930), there is definite evidence to believe that sheep grazed on the coastal parts of the South Western Districts of the Cape Province, suffer from an extreme deficiency of phosphorus. It was considered that Merino sheep kept in this area would provide suitable material for experimental work as with these animals no period of "acclimatisation" to a phosphorus deficiency would be necessary. Accordingly two classes of sheep, 40 yearling and 40 two-year old ewes, of a very uniform type,

were selected from a large flock belonging to a farmer in the Strandveld of the Bredasdorp District. That these animals were suffering from an extreme aphosphorosis is supported by the following facts:

1. The definite presence of osteophagia in many individuals.
2. The condition and state of the skeletal system. Numerous calluses could be felt on the ribs of some of the sheep. The fact that the bones were found to be weak and porous could be held responsible for the occurrence of these numerous fractures. A fairly detailed description of the physical and chemical properties of bones from these sheep will be found in the note of Bekker and Rossouw, referred to above.
3. The conspicuous under development and low condition of the animals. The group of yearling ewes averaged 46.1 lb., and the group of two-year old ewes averaged only 39.8 lb. when they became available for the experiment. The older ewes had been on the Strandveld for about 14 months, whereas their younger sisters had only been on this veld for about three months.
4. The low inorganic phosphorus content in the blood of the sheep. An analysis of the blood of the 80 experimental animals, made before phosphorus supplements were given, gave the following figures:—

Average of 39 two-year old ewes 3.3 mgm. P. per 100 c.c. blood
(extremes 1.8–4.9).

Average of 39 yearling ewes 4.0 mgm. P. per 100 c.c. blood
(extremes 2.8–5.3).

The average amount is definitely below what is considered normal, viz. ± 6.5 mgm. (Du Toit, Malan and Rossouw, 1930.)

5. Marked symptoms and sequelae of aphosphorosis in cattle grazing on the same pasturage.

ARRANGEMENT OF EXPERIMENT.

Bechuanaland veld is not considered very suitable for sheep. The grass grows tall and coarse and matures quickly, and in the past very heavy losses have occurred among sheep on account of severe verminosis; but as the grazing is very deficient in phosphorus, the conditions are, however, naturally fitted for studying the influence of feeding phosphorus supplements to sheep when grazed on a phosphorus deficient veld. A portion of the farm Armoedsvlakte, with no pans or standing open water and about 150 morgen in extent, was selected and enclosed with jackal-proof fencing. As it was found necessary to have two separate flocks, the camp had to be divided into two portions. Each of these smaller paddocks was provided with a water supply, and dosing pens and foot bath, etc., were erected in a convenient spot. As the flocks were not of equal size, in the one case 24 animals and in the other about 56, the paddocks were thus used alternately for grazing the two flocks, which were interchanged every week. In this way no doubt could arise as to the equality of grazing allowed to the animals in the various groups.

ARRANGEMENT OF EXPERIMENTAL GROUPS.

Prejudice or preference for placing the best animals in certain groups was avoided as numbers were assigned at random to the two classes of sheep, and then the following groups were arranged:—

Batch I.—(Two-year old ewes).

Group 1.—12 ewes (Nos. 1 to 12) allowed bonemeal lick, *ad lib.*, consisting of 75 per cent. bonemeal (\pm 22 per cent. P_2O_5) and 25 per cent. ordinary stock salt.

Group 2.—12 animals (Nos. 13 to 24) given free access to salt but no supplements, considered as controls of this batch.

Group 3.—5 animals (Nos. 25 to 29), free access to salt and each dosed daily, except Sundays, with $\frac{1}{2}$ oz. bonemeal.

Group 4.—5 animals (Nos. 30 to 34) treated as Group 3, but given $\frac{1}{4}$ oz. bonemeal.

Group 5.—5 animals (Nos. 35 to 39) treated as Group 3, but given $\frac{1}{8}$ oz. bonemeal.

Batch II.—(Yearling ewes).

Group 1.—12 ewes (Nos. 41 to 52) treated as Group 1, Batch I.

Group 2.—12 ewes (Nos. 53 to 64) treated as controls of Batch I.

Group 3.—7 ewes (Nos. 66 to 72) dosed daily except Sundays with $\frac{1}{2}$ oz. bonemeal to which 15 gm. Potassium iodide was added. (Later this was reduced to 0.75 gm.)

Group 4.—7 ewes (Nos. 73 to 79) dosed as the preceding group with 5 gm. NaCl + 15 gm. KI (later reduced to 0.75 gm.).

The experiment was commenced on 27th May, 1929.

No special reasons exist for compounding the bonemeal-salt lick in the proportions mentioned, excepting that farmers have been recommended to allow their sheep a lick containing these ingredients in these proportions. The amount of salt added will to a certain extent control the amount of lick consumed, and it was considered that sheep will consume about 5 gm. NaCl per day. When the lick contains 75 per cent. of bonemeal a sheep will probably consume \pm 15 gm. bonemeal per day. Numerous experiments have indicated that a daily dose of 3 oz. bonemeal suffices the requirements of bovines when kept on the Armoedsvlakte veld. Considering the corresponding size of sheep, a daily supplementary ration of 15 gm. bonemeal would most probably be adequate and even excessive.

When the experiment was commenced no information was available about the minimum optimum phosphorus requirements of sheep, therefore quantities of $\frac{1}{2}$ oz., $\frac{1}{4}$ oz. and $\frac{1}{8}$ oz. were prescribed to the animals in Groups 3, 4 and 5 of Batch I. It was thought that the experiment would yield data to indicate approximately the correct amount of bonemeal which should be allowed to sheep when grazing on veld so very deficient in phosphorus as that of Armoedsvlakte. It should be mentioned that difficulty was experienced in getting the animals to swallow the dose of bonemeal prescribed, and it often

happened that some sheep ejected their dose. In order to induce the sheep to swallow their dose of bonemeal a small quantity of *treacle* was added to the moist bonemeal. The quantity used was very small and it is not considered that the trace of treacle significantly influenced the results obtained.

Groups 3 and 4 of Batch II were included to observe the effects of iodine. In the one case the iodine was given where sufficient phosphorus was considered to be available and in the other case where aphosphorosis could be expected to develop. A daily dose of .15 gm. was decided upon, which after 5 months (15.10.29) was reduced to .075 gm. It should be mentioned that there is no reason to expect that an iodine deficiency exists in Bechuanaland, but as the importance and value of iodine supplements to farm stock has been so emphasized, it was considered worthwhile to ascertain the effects of additional iodine on sheep in this area. Orr and Leitch (1929) state that no definite information seems to be available regarding the iodine requirements of farm animals, nor is it definitely known what are sufficient or excessive doses. In their report it is also mentioned that Tinline on the one hand fed 100 to 170 mgm. KI while Torrance recommended much smaller doses, viz. 28 mgm. as a daily dose for sheep. Kelly (1925) found that a quantity of KI corresponding to .00019 per cent. I. of the food consumed failed to produce any demonstrable effect on the retention of calcium, phosphorus or nitrogen by the animals, whereas when the quantity of iodine was increased to .025 per cent. the effect becomes apparent. Considering that a sheep consumes $2\frac{1}{2}$ lb. grass per day, and not considering the amount of iodine naturally contained in the vegetation, it would, according to Kelly, be necessary to add about .037 gm. KI to the daily ration to get sheep to respond to iodine in the same way as pigs.

In view of the above information, the amount of iodine given to these experimental animals was high, although according to some of the authors quoted, *not excessive*.

The same general treatment was given to all the experimental animals. Besides being dosed throughout the experimental period with the prescribed amount of Government Wireworm Remedy, and during the summer months when ticks became troublesome and when the sheep were allowed to walk through a footbath containing a solution of arsenite of soda, the following additional general treatment was given to all the sheep:

2.5.29. To deal with nodular worms present, a rectal enema consisting of the following composition was administered:—

2 pints warm water;

$\frac{1}{2}$ oz. soda bicarbonate;

10 c.c. of Government Wireworm Remedy made up to the soluble form according to specifications.

10.10.29 and 23.10.29. About 2 weeks after shearing, the sheep were dipped in Cooper's double dipping powder to destroy keds.

6.12.29. All sheep were inoculated with bluetongue vaccine.

6.12.29. Abundant green grass was available as a result of which all the sheep were purging. Another rectal enema of the same composition as that used on 2.5.29 was administered.

EXPERIMENTAL RESULTS.

For convenience the results will be given under the following headings and discussed separately:—

1. The weight records—indicating loss or gain in condition, etc.
2. Lambing results.
3. Mortality in the different groups.
4. Amount of lick consumed.
5. Inorganic phosphorus determinations in the blood of experimental animals.
6. Observations on wool production.
7. The suitability of Bechuanaland veld as typified by Armoedsvlakte for Merino breeding.

(1) THE WEIGHT RECORDS.

The sheep were introduced on 27.5.29 into the experiment which was terminated at the end of September, 1930. The sheep were weighed every fortnight and care was taken to allow the animals to drink their fill before each weighing.

The weight records are summarized in Tables 7 and 8 (vide appendix) and in the following table the averages of those results are given:—

TABLE No. 1.
Average weight (lb.) of sheep in various groups.

Group No.	Initial Weight. 27.5.29.	Weight. 21.12.29.	Gain.	Maximum Weight During Exptl. Period.	Maximum Gain.	Weight at End of Exptl. Period (Sep., 1930).
<i>BATCH I.</i>						
Group 1.						
B.M. Lick.....	42.5	60.1	17.7	84.2	41.8	75.0
Group 2.						
Controls.....	42.3	51.1	7.7	71.2	29.0	61.0
Group 3.						
$\frac{1}{8}$ -oz. B.M.....	40.0	57.6	15.6	79.4	39.2	66.2
Group 4.						
$\frac{1}{4}$ -oz. B.M.....	37.8	49.4	11.6	71.8	34.0	65.0
Group 5.						
$\frac{1}{2}$ -oz. B.M.....	41.8	60.8	19.0	81.8	41.0	69.7
<i>BATCH II.</i>						
Group 1.						
B.M. Lick.....	51.1	65.2	14.1	85.4	35.3	73.4
Group 2.						
Controls.....	45.6	54.6	9.0	74.7	28.8	62.3
Group 3.						
B.M.—K1.....	45.4	55.8	10.4	71.7	26.6	57.4
Group 4.						
Salt—K1.....	48.7	58.7	9.4	76.7	26.4	63.3

Two rams were admitted to the ewes on 15.10.29, and the first lamb was born on 8th May, 1930. The gestation period and subsequent lactation considerably interfered with the weights of some individuals, therefore only the weights of the sheep in the different groups before any of the ewes came into lamb can be utilized for strict comparison. The weight records made on 21.12.29 are thus given and the gain which the animals made over the period of approximately 7 months. For further interest the maximum weight and gain attained by the ewes during the whole experimental period and their weights at the termination of the experiment are included.

Special attention is drawn to the following features brought out by these records:—

1. The low condition, especially of Batch I, of the sheep at the commencement of the experiment is indicative of the aphosphorosis which develops in sheep grazing on the Strandveld in Bredasdorp.

2. The sheep receiving bonemeal supplements showed greater increase in weight than the controls and maintained this better condition throughout the experimental period. This is especially the case in Batch I, which were those sheep considered to have been in a greater degree of phosphorus deficiency. The relative greater improvement in weight of the bonemeal-fed animals when compared with the controls is not as great as was expected, especially in view of the phenomenal increase obtained with cattle by giving the phosphorus containing supplements on this farm. It should, however, be borne in mind that the sheep were already approaching maturity and the difference in weight mainly reflects an improvement in condition brought about by the feeding of the phosphorus containing supplement.

3. The results obtained in Groups 3, 4 and 5 of Batch I do not clearly indicate the optimum amount of phosphorus supplement which is required by sheep grazing on the phosphorus deficient veld of Arnoedsvlakte. The numbers included in these groups are too small to permit absolute and final conclusions to be made. It appears that even such a small quantity as $\frac{1}{8}$ oz. fed daily had beneficial effects, although the best results seem to have been achieved where $\frac{1}{2}$ oz. was given.

4. A few individuals which were allowed the bonemeal lick showed little increase in weight, e.g. Nos. 9 and 45. These animals were perhaps more heavily infected with internal parasites, or their comparative low condition would seem to indicate that they did not "take to" the lick as well as the other sheep. No direct observations were made to confirm this, but it will not at all be surprising if this is found to be true, for it is known that in cattle osteophagia remains in abeyance in some individuals in spite of the presence of other advanced symptoms of a phosphorus deficiency. The fact that pica is only noted in sheep under extreme conditions of phosphorus deficiency seems to indicate that the sheep is apparently much more selective and fastidious in its feeding habits than the bovine. This will support the idea that some sheep will not readily take a lick containing bonemeal. The salt usually contained in such licks enhances the palatability for sheep.

5. The sheep receiving iodine supplements showed about the same increase in weight as the control groups. It would seem that the iodine supplement, administered in this amount might even have an adverse effect in this respect, as no corresponding improvement was made in Group 3 when compared to Group 2 of this Batch and the various bonemeal-fed groups of Batch 1.

(2) LAMBING RESULTS.

Du Toit and Bisschop (1929) have drawn attention to the fact that fertility of cattle grazing on a phosphorus deficient pasturage is remarkably improved when the deficiency is corrected with a supplement such as bonemeal. Guilbert and Hart (1929) found that under-nutrition stops the oestrus cycle in white rats and low protein and phosphorus intake tend to stop or at least greatly lengthen it. Some data are available from this field experiment to indicate the influence of a phosphorus supplement on the fertility of the smaller ruminant when grazed on a pasturage known to be deficient in this element. Two rams of known fertility were run continuously with the ewes for different periods. In order to exclude the individual influences of the rams, each was kept alternately every week with the two flocks of ewes. The rams were first admitted on 15.10.29 and kept with the ewes for six weeks (to 31.12.29). During this period only a few ewes came into lamb, so the rams were again admitted to run with the ewes from 19.4.30 to 19.5.30. Quinlan and Maré (1931) have established that in South Africa oestrus occurs regularly in normal Merino ewes at intervals of 16-18 days throughout the entire year. The "service periods" allowed in the above cases were sufficient to ensure that all ewes which developed oestrus would be served. Table No. 2 has been prepared to illustrate the number of ewes in the different groups which came into lamb (as indicated by lambs born or aborted).

TABLE No. 2.
Number of lambs born or aborted.

Nature of Supplement.	Batch No.	Group No.	Total No. Ewes Available to Lamb.	Total No. Lambs Born or Aborted.	Percentage Lambs Born from Available Ewes.
Bonemeal.....	I	1	11	4	36.4
	I	3	5	4	80.0
	I	4	5	2	40.0
	I	5	5	3	60.0
	II	1	12	7	58.3
Total.....	—	—	38	20	52.6
No supplement.....	I	2	10	2	20.0
	II	2	12	5	41.6
Total.....	22	7	31.8
Iodine and bonemeal.....	II	3	7	1	14.3
	II	4	7	6	85.7
Total.....	14	7	50.0

From the results it would seem that the addition of phosphorus brought about an improvement in condition in the sheep and the fertility of those ewes was beneficially influenced. The effect of the iodine was noteworthy. Five of the seven ewes which came into lamb aborted and the other two lambs were so weak that they died shortly after birth. It seems safe to assume that iodine given in this amount (0.75 gm. per day) is detrimental to pregnant ewes. This is in accordance with the findings of Du Toit and Malan (1932) and Steyn (1931), the latter author having fed iodine to goats.

(3) MORTALITY IN THE DIFFERENT GROUPS.

In the cattle breeding experiments described by Du Toit and Bisschop (1929), remarkable results relating to the "incidence of disease" under the conditions of sufficient and deficient phosphorus are recorded. Over a period of three years the mortality in groups of cattle grazed on a phosphorus deficient veld was 66 per cent., whilst in the groups which received a phosphorus supplement (bonemeal) it was only 8.9 per cent. In this sheep experiment the numbers of deaths which occurred in the various groups over the experimental period are given in Table 3.

TABLE NO. 3.

Mortality in different groups.

Nature of Supplement.	Batch No.	Group No.	Number in Group.	Number of Deaths.	Percentage.
Bonemeal.....	I	1	12	1	8.3
	I	3	5	0	0
	I	4	5	0	0
	I	5	5	0	0
	II	1	12	1	8.3
Total.....			39	2	5.1
No Supplement.....	I	2	12	4	33.3
	II	2	12	5	41.6
Total.....			24	9	37.5
Iodine.....	II	3	7	1	14.3
	II	4	7	0	0
Total.....			14	1	7.1

The mortality is distinctly higher in the groups which received no supplement. Most of the deaths which occurred could be associated with infestation with internal parasites (see appendix Table 6 for details). Mönnig (1924) found that it is much more difficult to provoke trichostrongylosis artificially in sheep which are in good condition than in ill-nutritioned animals in poor condition. The animals receiving the bonemeal supplement could be considered to have been on a better plane of nutrition and were naturally better equipped to withstand the injurious effects of verminosis than the controls.

(4) THE AMOUNT OF LICK CONSUMED.

From the 27th May, 1929, to 30th September, 1930, the animals which received the bonemeal lick consumed 870 lb. of the material. For most of the time there were 24 animals in this flock and 23 for a limited period. It may, therefore, be considered that on an average each animal consumed $\frac{870 \times 16}{24 \times 491} = 1.2$ oz. per day over that period. As the lick consisted of 75 per cent. bonemeal, each animal took .9 oz. bonemeal daily. It was found that the sheep did not consistently consume the same amount of lick, as more of the lick was taken during the dry winter months than during the summer months, when the grazing was green. Quantities of 10 lb. was added to the trough when nearly empty. During the winter months this quantity had to be added every 3 days, the sheep were, therefore, taking more than 2 oz. each daily. During the summer months, when the grazing was green, the troughs had to be replenished every sixth or seventh day, so that they were now taking a little less than 1 oz. per day. Considering the present price of bonemeal, £8 per ton, it would cost a farmer about 1s. 6d. to 2s. per animal per annum to feed bonemeal to sheep under the same conditions as this experiment was carried out. The monetary return from ordinary flock Merinos does not seem to warrant investing this amount of money in a lick, and means and methods should be devised to bring the cost of a phosphorus supplement, in parts of the country where it is necessary, within more economical limits.

(5) INORGANIC PHOSPHORUS DETERMINATIONS IN THE BLOOD OF THE EXPERIMENTAL ANIMALS.

The value of determining the inorganic phosphorus fraction of the blood of ruminants as a method of diagnosing and studying aphosphorosis has been emphasized by Du Toit, Malan and Rossouw (1930). Five sheep in each experimental group were bled at intervals and the inorganic phosphorus determined according to the method described by Green (1928). The average amount present in the blood will be found in Table No. 4.

TABLE NO. 4.
Inorganic Phosphorus in the Blood of Experimental Animals
 (mgm. per 100 c.c.).

Group No.	15.5.29	—	31.10.29	28.12.29	23.1.30	22.2.30	20.3.30	22.4.30	21.5.30	20.6.30	21.7.30	21.8.30	22.9.30
<i>BATCH I.</i>													
Group 1 (B.M. lick).....	2.9	5.8	8.0	5.9	6.6	4.7	4.7	5.7	6.8	7.7	7.8	7.7	6.5
Group 2 (controls).....	3.1	3.6	3.8	4.7	4.6	3.9	4.3	4.5	3.6	3.8	5.5	4.8	4.5
Group 3 ($\frac{1}{2}$ -oz. B.M.)....	3.7	6.2	6.8	5.9	7.2	5.2	6.8	5.6	5.6	6.5	7.1	7.4	6.0
Group 4 ($\frac{1}{4}$ -oz. B.M.)....	2.9	6.0	7.4	3.1	6.3	4.8	5.7	5.7	5.0	5.5	7.0	7.2	6.3
Group 5 ($\frac{1}{2}$ -oz. B.M.)....	3.7	6.2	7.6	6.4	6.2	4.6	5.4	5.1	5.5	6.1	7.5	7.3	5.9
<i>BATCH II.</i>													
Group 1 (B.M. lick).....	4.5	5.5	6.9	5.7	5.6	4.6	5.1	5.6	4.8	6.0	7.2	6.5	6.8
Group 2 (controls).....	3.8	3.8	3.5	4.6	6.1	3.9	3.9	4.2	3.8	4.8	4.6	5.0	4.0
Group 3 (B.M. + KI)....	4.0	5.6	6.8	6.8	5.8	4.9	6.6	6.3	6.0	7.3	7.5	7.6	6.5
Group 4 (salt + KI)....	3.7	3.6	3.8	5.9	4.9	3.8	3.8	4.5	3.9	4.6	4.7	4.9	5.2

The controls of Batches I and II show lower values than the animals receiving bonemeal. In Group 3 of Batch II (NaCl+KI) the values correspond to those of the controls and the iodised-bonemeal group correspond very closely to the animals receiving the lick. In Batch I there is no distinct difference in the amount of inorganic phosphorus present in the blood of the groups *receiving various amounts of bonemeal*. These results seem to indicate that in sheep low phosphorus content of the diet can be diagnosed by means of these analyses, but the data accumulated in this experiment gives no indication of the relative amount of phosphorus administered to the animals in the different groups. Du Toit, Malan and Rossouw (1930) state that the inorganic phosphorus level of the blood may be taken as being indicative of the *degree of phosphorus deficiency* of the animal, thus it would seem that a supplement of $\frac{1}{2}$ oz. of bonemeal per diem provides sheep with a sufficient amount of phosphorus under Armoedsvlakte grazing conditions.

(6) OBSERVATIONS ON WOOL PRODUCTION.

The conditions under which the experiment was conducted were not considered suitable for making a detailed study of the wool produced by the sheep. It is believed that a deficiency of phosphorus will lead to the wool fibres becoming more attenuated. Theiler and Orr (1929) state that in parts of Tasmania where a phosphorus deficiency is present in an exaggerated measure in cattle, the sheep running on the same pasture were producing the finest wools. Although no conclusive direct experimental data is at the moment available, there is much indirect evidence which supports this idea. The writer has also observed that very fine wool is produced by sheep grazed on the coastal farms of the South-Western Districts of the Cape Province where there is undisputable evidence of an extreme phosphorus deficiency. It should be borne in mind that the phosphorus deficiency results in a state of ill-nutrition of the animal body, and it is this unbalanced plane of nutrition which leads to the production of wool fibres of smaller cross-sectional area. A phosphorus deficiency is only one of the many factors which may be responsible for the fining affect on the wool. Some of these factors are:—

1. General malnutrition, e.g. during droughts when there is a general scarcity of food or during the winter periods of the summer rainfall areas of the Union when the protein content of the vegetation is low. These two factors alone are responsible for the imparting of distinct qualities, as recognized by the wool trade, on South African wool. When, for instance, a general and prolonged drought is experienced in South Africa, wool buyers report favourably upon the fineness of the wool produced during such a season.

2. Diseased conditions, especially those accompanied by high fever, or where a severe anaemia is provoked. The attenuation in such instances might become so marked that a distinct "break" occurs in the wool with the result that the wool "falls out". It is well known that sheep may lose their wool after having passed through a severe attack of bluetongue (high fever) or severe verminosis (anaemia) or trypanosomiasis (fever and anaemia).

3. Physiological conditions which result in a lowering of the nutritional plane of the body, e.g. pregnancy and lactation, senile decay, athyroidism (after thyroidectomy), etc.

In view of the above it would have been very difficult to utilize the wool produced by the sheep in this experiment for making a systematic study of the effect of the phosphorus supplements on the various attributes of the fibres. Too many complicating factors were present, e.g. infestation by intestinal parasites (especially oesophagostomes), and the gestation and lactation of some of the ewes. Messrs. du Toit and Pepler, sheep and wool experts, kindly examined the wool on the sheep. In a general way it appeared as if the wool produced by the sheep receiving the bonemeal supplements was sounder, but it was impossible to consider that the wool from the control groups was generally distinctly finer than the phosphorus supplemented groups. The sheep were shorn twice during the course of the experiment, and the entire fleeces weighed. It is realized that this is not a good method of ascertaining the *amount* of wool produced by a sheep, as undoubtedly the only satisfactory method is to base the amount on a soured and conditioned basis. In any case the following were the average weights of the fleeces.

TABLE No. 5.
*Average Amount (Kgm.) of Wool Shorn from Animals in
Different Groups.*

Group No.	21.9.29. (+ 10 months wool) Kgm.	26.9.30. (1 year wool) Kgm.	Difference in Amount Shorn Kgm.
<i>BATCH I.—TWO YEAR OLD EWES.</i>			
Group 1.—Bonemeal lick.....	2.456	3.616	1.160
Group 2.—Controls.....	2.456	3.554	1.098
Group 3.— $\frac{1}{8}$ -oz. bonemeal.....	2.464	3.487	1.023
Group 4.— $\frac{1}{4}$ -oz. bonemeal.....	2.492	3.378	0.886
Group 5.— $\frac{1}{2}$ -oz. bonemeal.....	2.554	3.452	0.898
<i>BATCH II.—YEARLING EWES.</i>			
Group 1.—Bonemeal lick.....	2.847	3.570	0.723
Group 2.—Controls.....	2.534	7.170	0.636
Group 3.— $\frac{1}{2}$ -oz. bonemeal—KI.....	2.267	2.804	0.537
Group 4.—5 gm. NaCl—KI.....	2.507	3.053	0.546

Considering the errors encountered in arriving at these fleece weights, no great significance could be attached to the apparently increased amount of wool produced by some groups.

(7) THE SUITABILITY OF BECHUANALAND VELD AS TYPIFIED BY
ARMOEDSVLAKE FOR MERINO-BREEDING.

It was mentioned in the introduction that apart from observing the influence of phosphorus and iodine supplements when given to sheep, the experiment was also designed to give some indications whether Merino sheep breeding could be successfully developed in Bechuanaland. In the past various attempts have been made to introduce better bred sheep on to this veld, but such undertakings have not been considered successful. The sheep in this experiment were kept under what could be considered improved conditions, but these

arrangements were essentially practical and could be adopted by the ordinary farmer. In spite of the care bestowed on these animals, it cannot be claimed, however, that the general results in any one group were so good that a policy of encouraging and developing Merino sheep farming in Bechuanaland could be embarked upon. Under the circumstances it seems advisable to draw attention to a few factors which are important and adversely affect the possibilities of successfully farming with sheep, especially woolled ones, in this area.

The Nature of the Vegetation.—Armoedsvlakte veld, which is quite typical of a large portion of Bechuanaland, does not appear to be very well suited for woolled sheep. In the first place a fair amount of shrubs and low bushes are to be found, especially the “Rosyntjie bos” (*Grewia cana*). Sheep are wont to graze between these bushes and even underneath them and a large amount of wool is torn out. In the second place the tall growing varieties of grasses are unsuitable for the grazing habits of sheep. By heavy stocking with cattle these varieties become somewhat suppressed, but with this change in the veld “steekgras” (*Aristida congesta*) becomes dominant or subdominant, and this grass has the well-known deleterious effects on woolled sheep. The presence of so much “steekgras” in parts of Bechuanaland is a very important factor when considering the potentialities of that country for the breeding of Merinos.

Verminosis.—On numerous occasions the writer and others have found that the alarming mortality which has occurred in sheep in parts of Bechuanaland could be ascribed chiefly to infestation with stomach and intestinal parasites. The worms mainly responsible being wireworm (*Haemonchus contortus*), nodular worm (*O. columbianum*), hookworm (*Gaigeria pachyscelis*) and bankrupt worm (*Trichostrongylus* spp.).

Although the sheep in this experiment were systematically treated for worms according to available methods, and although recommended hygienic measures were adopted, e.g. free grazing, clean water supply, avoidance of dangerous open water, etc., they gradually became very heavily infected with nodular worms (*Oesophagostomiasis*), and in those sheep which died, a very heavy infection was present. The mortality was certainly lower in the groups dosed with bonemeal when compared with the controls, but towards the termination of the experiment the condition of some of the animals, including cases receiving phosphorus supplements, was rapidly falling, and other well-known symptoms of worm infection were becoming manifest.

It would seem from the observations made in this experiment that the addition of phosphorus supplements to the deficient diet of the sheep is of value and should be arranged, but this correction will by no means lead to the final solution of all the evils and troubles encountered by the sheep farmer in Bechuanaland. Systems of veld management may modify the present detrimental botanical features of the grazing for sheep, but it is considered that intestinal parasitic diseases, especially infestation with the nodular worm, is the most serious menace which must be overcome before sheep farming can form an important part of the activities of pastoral Bechuanaland.

SUMMARY.

1. Experimental work with phosphorus and iodine supplements given to sheep under veld conditions known to be deficient in phosphorus is described.

2. Sheep which had previously been kept on the Strandveld of the Bredasdorp District, Cape Province, and where aphosphorosis is extreme, were used.

3. A bonemeal-salt lick consisting of one part salt and three parts bonemeal was allowed *ad lib.* to groups. Others were dosed with $\frac{1}{8}$, $\frac{1}{4}$ and $\frac{1}{2}$ oz. of bonemeal. Two groups received a supplement of 15 gm. KI (after 5 months reduced to 0.75 gm.) per day; in the one case this supplement was given with $\frac{1}{2}$ oz. bonemeal and in the other with 5 gm. ordinary salt. Appropriate control groups were kept.

4. The sheep receiving the bonemeal supplement, as indicated by fortnightly weighings, showed better development and condition than the controls after 6 months' feeding. In most cases they showed approximately double the gain made by control groups.

5. The groups dosed with iodine behaved more or less like the controls.

6. In the bonemeal supplemented groups more ewes became pregnant and lambed than in the control groups. Of the seven animals which became pregnant in the iodised groups five aborted, and the other two lambs born were weaklings and died shortly after birth. It is considered that the doses administered (*viz.* 15 and 0.75 gm.) are excessive and even toxic when given over a prolonged period.

7. The mortality, especially from verminosis, was considerably higher among the control groups (9 out of 24) than among the groups receiving bonemeal (3 out of 39).

8. The sheep given the bonemeal-salt lick consumed on an average 1.2 oz. lick daily (the maximum consumption during the winter months was more than 2 oz. per day, during summer less than 1 oz. was taken). Considering bonemeal to cost £8 per ton, it will cost about 1s. 6d. per sheep per annum to give this amount. On considering the monetary returns from Merino sheep, it seems as if this is an uneconomical amount to invest in a lick, and methods should be devised to reduce the cost to more economical limits.

9. The inorganic phosphorus content of the blood of the experimental animals was determined at intervals during the experimental period. It was found that the inorganic phosphorus in the blood of the control animals remained lower than in the bonemeal-fed groups. The administration of iodine with bonemeal did not appear to affect the inorganic phosphorus content of the blood.

10. Under the conditions which this experiment was carried out no well-defined differences in the amount and quality of wool produced could be determined, but other complicating factors, *e.g.* verminosis, apparently masked the effect of the supplements.

11. It is impossible from these series of experiments to conclude what optimum minimum quantity of phosphorus supplement is required by sheep on the phosphorus deficient veld as typified by Armoedsvlakte pasturage. It appears, however, as if even such a small daily dose as $\frac{1}{2}$ oz. bonemeal gives beneficial results.

12. It is considered that other conditions, apart from phosphorus deficiency, especially the nature of the vegetation and the fact that sheep are so subject to infestation with intestinal parasites, makes it very problematical whether Merino sheep breeding can be successfully developed in Bechuanaland, especially where the veld conditions are similar to those of Armoedsvlakte. Successful practical measures and methods to cope with some of these problems encountered with sheep farming in this area are at the moment not available.

BIBLIOGRAPHY.

- BEKKER, J. G., AND ROSSOUW, S. D. (1930). A Note on Some Conditions in Sheep in the Strandveld of the Bredasdorp District. *16th Rept. Dir. Vety. Serv. Un. of S.A.*, pp. 293-300.
- DU TOIT, P. J., AND BISSCHOP, J. H. R. (1929). The Breeding of cattle on Phosphorus Deficient Veld. *15th. Rept. Dir. Vety. Serv. Un. of S.A.*, pp. 1059-1168.
- DU TOIT, P. J., AND MALAN, A. I. (1932). The rôle of Iodine in the Nutrition of Sheep. *This Report*.
- DU TOIT, P. J., MALAN, A. I., AND ROSSOUW, S. D. (1930). Phosphorus in the Sheep Industry. *16th Rept. Dir. Vety. Serv. Un. of S.A.*, pp. 313-326.
- GUILBERT, H. R., AND HART, G. H. (1929). Calf. Expt. St. unpublished data (Communicated by G. H. Hart Reports 11th *International Veterinary Congress*, 1930).
- GREEN, H. H. (1928). The Determination of Phosphorus Compounds in Blood. *Jnl. Agric. Sc., Vol. 18, Pt. 3*, pp. 372-375.
- KELLY, F. C. (1928). The Influence of small Quantities of Potassium Iodide on the Assimilation of Nitrogen, Phosphorus and Calcium in the Growing Pig. *Biochem. Jnl.*, Vol. 19, No. 4.
- MÖNNING, H. O. (1924). Life History of *Trichostrongylus motabilis* and *T. rugatus* of Sheep in South Africa. *11th & 12th Repts. Dir. of Vety. Ed. & Res. U. of S.A.*, pp. 231-254.
- ORR, J. B., AND LEITCH, I. (1929). Iodine in Nutrition. *Special Report No. 123, H.M. Stationery*.
- QUINLAN, J. B., AND MARE, G. S. (1931). The Physiological Changes in the Ovary of the Merino in South Africa and their Application in Breeding. *17th. Rept. Dir. of Vety. Serv. U. of S.A.*, pp. 663-706.
- STEYN, D. G. (1931). Investigations into the Cause of Alopecia (Kaalsiekte) in Kids and Lambs. *17th. Rept. Dir. Vety. Serv. U. of S.A.*, pp. 729-770.
- THEILER, A., AND ORR, J. B. (1929). The Health and Nutrition of Animals H. J. Green, Government Printer, Melbourne.

APPENDIX.

TABLE No. 6.

*Details of Deaths in Various Groups.**Batch I.*

Group 1. (Bonemeal lick):	
Sheep No. 2.	Died 2.9.29, found dead, cause unknown.
Group 2. (Controls):	
Sheep No. 16.	Died 10.12.29, killed by jackal.
" " 19.	" 28.8.29, verminosis.
" " 20.	" 30.8.29, "
" " 23.	" 29.9.29, "

Batch II.

Group 1. (Bonemeal lick):	
Sheep No. 47.	Died 27.8.30, cause of death unknown.
Group 2. (Controls):	
Sheep No. 53.	Died 15.9.30, inability to lamb.
" " 55.	" 21.6.30, verminosis.
" " 60.	" 1.10.30 "
" " 63.	" 29.9.30 "
Group 3. (Bonemeal and KI).	
Sheep No. 68.	Died 29.9.30, verminosis.

TABLE 7.

Summary Weight (lb.) Records of Sheep Batch II.—Yearling Ewes.

Group No.	Initial Weight, 27.5.29.	Weight, 21.12.29.	Gain.	Maximum Weight During Exptl. Period.	Maximum Gain During Exptl. Period.	Weight at Termina- tion of Experiment 13.9.30.
Group 1. B.M. lick.						
41.....	53	66	13	95	42	73
42.....	50	67	17	86	36	84
43.....	59	68	9	103	44	98
44.....	43	54	11	74	31	67
45.....	50	53	3	65	15	52
46.....	45	60	15	65	20	59
47.....	50	72	22	96	46	69
48.....	52	72	20	98	46	98
49.....	50	62	12	86	36	76
50.....	48	64	16	81	33	63
51.....	57	69	12	87	30	87
52.....	56	75	19	101	45	68
Averages.....	51.1	65.2	14.1	85.4	35.3	73.4
Group 2. Controls.						
53.....	48	50	2	62	14	60
54.....	44	57	13	75	29	48
55.....	44	51	6	64	20	—
56.....	49	60	11	85	36	55
57.....	44	52	8	67	23	59
58.....	44	51	7	69	25	62
59.....	51	58	7	90	39	81
60.....	36	43	7	62	26	53
61.....	42	55	13	80	38	71
62.....	51	61	10	89	38	65
63.....	50	63	13	87	37	75
64.....	44	57	13	68	24	53
Averages.....	45.6	54.6	9.0	74.7	28.8	62.3
Group 3. Iodised B.M.						
66.....	43	46	3	78	35	64
67.....	49	57	8	71	22	59
68.....	36	48	12	53	17	41
69.....	51	66	15	68	17	57
70.....	39	49	10	64	25	53
71.....	43	61	18	76	33	53
72.....	57	64	7	92	37	75
Averages.....	45.4	55.8	10.4	71.7	26.6	57.4

TABLE 7 (*continued*).*Summary Weight (lb.) Records of Sheep Batch II.—Yearling Ewes.*

Group No.	Initial Weight, 27.5.29.	Weight, 21.12.29.	Gain.	Maximum Weight During Exptl. Period.	Maximum Gain During Exptl. Period.	Weight at Termination of Experiment 13.9.30.
Group 4. Iodised Salt.						
73.....	48	52	4	69	21	49
74.....	44	53	9	66	22	51
75.....	40	52	12	67	27	57
76.....	53	58	5	79	26	66
77.....	52	67	15	85	33	75
78.....	56	70	14	92	36	74
79.....	48	55	7	78	30	71
Averages.....	48.7	58.1	9.4	76.7	26.4	63.3

TABLE 8.

Summary Weight (lb.) Records of Sheep Batch I.—Two-year old Ewes.

Group No.	Initial Weight, 27.5.29.	Weight, 21.12.29.	Gain	Maximum Weight During Exptl. Period.	Maximum Gain During Exptl. Period.	Weight at Termination of Experiment 13.9.30.
Group I. B.M. lick.						
1.....	42	65	23	100	58	97
2.....	44	—	—	—	—	—
3.....	39	58	19	93	54	90
4.....	46	67	21	85	39	85
5.....	43	68	25	89	46	86
6.....	58	87	29	119	61	94
7.....	42	60	18	87	45	71
8.....	41	58	17	73	32	66
9.....	42	46	4	74	32	68
10.....	36	46	10	61	25	44
11.....	35	52	17	67	32	58
12.....	42	54	12	78	36	70
Averages.....	42.5	60.1	17.6	84.2	41.8	75.4

TABLE 8 (*continued*).
Summary Weight (lb.) Records of Sheep Batch I.—Two-year old Ewes.

Group No.	Initial Weight, 27.5.29.	Weight, 21.12.29.	Gain.	Maximum Weight During Exptl. Period.	Maximum Gain During Exptl. Period.	Weight at Termination of Experiment 13.9.30.
Group 2. Controls.						
13.....	42	56	14	73	31	69
14.....	36	44	8	64	28	58
15.....	46	44	2	61	15	48
16.....	39	—	—	—	—	—
17.....	43	49	6	64	21	56
18.....	47	61	4	88	41	65
19.....	44	—	—	—	—	—
20.....	45	—	—	—	—	—
21.....	43	50	7	81	38	71
22.....	42	51	9	78	36	65
23.....	39	50	11	64	25	55
24.....	42	55	13	68	26	62
Averages.....	42.3	51.1	7.7	71.2	29.0	61.0
Group 3. $\frac{1}{8}$ oz. B.M. dosed						
25.....	39	63	14	78	39	60
26.....	43	61	18	88	45	76
27.....	45	56	11	83	38	60
28.....	34	54	10	70	36	63
29.....	40	55	15	78	38	72
Averages.....	40.0	57.6	15.6	79.4	39.2	66.2
Group 4. $\frac{1}{4}$ oz. B.M. dosed.						
30.....	36	45	9	70	34	65
31.....	32	40	8	65	33	58
32.....	45	56	11	79	34	75
33.....	38	53	15	80	42	70
34.....	38	53	15	65	27	57
Averages.....	37.8	49.4	11.6	71.8	34.0	65.0
Group 5. $\frac{1}{2}$ oz. B.M. dosed.						
35.....	43	63	20	80	37	74
36.....	47	65	18	108	61	75
37.....	40	61	21	83	43	68
38.....	42	59	17	77	35	66
39.....	37	56	19	76	29	64
Averages.....	41.8	60.8	19.0	84.8	41.0	69.7

Studies on Mineral Metabolism, XXIV.

“On the Administration of Phosphorus to Animals through their Water Supply.”*

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INTRODUCTION.

- I. Present methods of administering phosphorus to animals and remarks on each method.
 - (a) Mixing phosphorus-containing supplements with food.
 - (b) Handfeeding.
 - (c) Licks:
 1. “ Small-box system ”.
 2. Licks given *ad lib*.
 - (d) Correcting P_2O_5 content of the soil.
- II. Availability to the animals of phosphorus in materials commonly used as phosphorus supplements.
- III. Suggested new method of administering P_2O_5 to animals in the drinking water, with cursory remarks on the water supply of stock in South Africa.

ARRANGEMENT OF EXPERIMENTAL WORK.

- I. Arrangement of water supply to experimental animals.
- II. Paddocks.
- III. Animals used and their general treatment.
- IV. The choice and amount of materials used:
 - (a) The phosphorus-containing supplements.
 - (b) The addition of H_2SO_4 to the water for complete solution of phosphates.
 - (c) The addition of a very small quantity of copper sulphate to inhibit the growth of algae.

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RESULTS OBTAINED.

- I. Amount of water consumed.
- II. The weight of experimental animals.
- III. Osteophagia in different groups.
- IV. Clinical observations:
 - (a) Styfsiekte.
 - (b) Lamsiekte.
 - (c) Anaplasmosis.
 - (d) Digestive Disturbances.
- V. Inorganic Phosphorus determinations in the blood of animals.

A SUGGESTION FOR THE ARRANGEMENT OF THE WATER SUPPLY FOR THE ADOPTION OF THE METHOD OF ADMINISTERING P. TO ANIMALS IN THE WATER SUPPLY.

ECONOMICAL AND PRACTICAL CONSIDERATIONS OF THE METHOD.

SUMMARY AND CONCLUSIONS.

ACKNOWLEDGMENTS.

REFERENCES.

APPENDIX.

INTRODUCTION.

SINCE Theiler (1920) established the fact that a phosphorus deficiency in the soil and vegetation is a link in the etiology of lamsiekte, phosphorus containing substances, such as bonemeal, are being extensively fed to cattle in those areas where such a deficiency exists. This has revolutionized cattle farming in parts of South Africa. In the pastoral districts of Bechuanaland cattle husbandry was a very precarious industry on account of the ravages of lamsiekte, and when once the value of phosphorus to stock had been demonstrated, farmers enthusiastically adopted the practice of supplying their cattle with it, the medium generally favoured being bonemeal. Not only was the dreaded disease lamsiekte overcome, but the addition of phosphorus to the deficient diet of the animals resulted in a remarkable improvement in the development, condition, productivity and fertility of their cattle. Formerly herds representing heterogeneous types of native scrub cattle struggled for an existence in those parts. Attempts either to keep modern improved breeds, or to improve the undesirable types of cattle with pure-bred bulls, proved of little value, the pasturage, chiefly on account of its limitation in phosphorus, being of

such a nature that it was impossible for these highly specialized breeds to develop and exhibit their characteristics. Such animals kept on this deficient veld assumed a scrub-like appearance and were no better than the indigenous native breeds. As soon as this outstanding limiting factor was removed by the administration of a phosphorus supplement to the animals in the form of bonemeal, they responded in a remarkable way to the improved conditions. Farmers can now successfully keep grades and even pure-bred cattle on this veld and such animals exhibit their specialized breed characteristics: the phenotype comes to be a truer representative of the genotype. Bonemeal has indeed become an indispensable stock-food in those districts. The present *methods*, however, of administering phosphorus to animals have their shortcomings.

I. PRESENT METHODS OF ADMINISTERING PHOSPHORUS TO ANIMALS AND REMARKS ON EACH METHOD.

(a) *Mixing phosphorus containing supplements with the food.*

Where cattle are kept under intensive methods of farming and are given supplementary feeds, bonemeal, di-calcium phosphate, etc., can be added to the feed and in this way the animals will receive an adequate supply of phosphorus. This method is not laborious and presents no difficulties in the case of stabled animals. In South Africa, however, cattle are seldom housed, being usually entirely veld-reared, especially in those districts where there is a marked deficiency of phosphorus. Under these circumstances the phosphorus-containing supplements have to be administered by other methods.

(b) *Hand-feeding.*

This is the method developed and favoured by Theiler and his co-workers. All the animals must be collected daily and put through a crush in batches. As they pass through the crush their mouths are opened and the bonemeal, etc., administered by means of a basting spoon. The method has been described in great detail by Du Toit and Bisschop (1929), and it will therefore not be necessary to describe it again. Farmers, especially those having moderately-sized properties, have successfully adopted it. Some of the disadvantages are enumerated below:

(1) In order to obtain the maximum benefit from giving a supplementary ration of phosphorus, Theiler, Green and Du Toit (1924) advised the daily dosing of animals with the phosphorus-containing substance. This means that all cattle have to be collected and put through a crush every day. A considerable amount of labour is entailed, and in many instances on large farms, additional stock horses have to be kept.

(2) The continual herding and collecting of animals results in unnecessary and destructive trampling of the veld. The Drought Commission in its report (1923), stresses the deleterious effects of excessive trampling of the veld, as undoubtedly this is one of the primary causes of veld-deterioration in South Africa.

(3) A certain amount of training is necessary to accustom the animals to go through the crush and to allow their mouths to be opened forcibly. It often happens that animals are injured, and some of them never become tractable.

(4) A system such as this continually interferes with the natural grazing habits of the animals. On farms the cattle are usually collected in the very early morning, which, especially during the hot summer, is the time the animals like to graze. If this early grazing is prevented the animals are forced to graze during the hot part of the day, the time when they seek shade and rest.

(5) A considerable amount of energy is expended by the cattle whilst being driven to the part of the farm where the dosing is carried out. If this enforced expenditure of energy could be avoided, the animals would put on more condition. In Bechuanaland it may be considered that on an average animals have to be driven at least 2 miles each way to and from their camps in order to be dosed.

During droughts, i.e. during the period of scarcity in grazing, animals are in greatest need of the deficient element phosphorus, and at the same time they can least afford the loss of energy in their daily drives. In practice this means that the highly essential phosphorus supplement is unfortunately obtained by the animal at a cost in body katabolism, which it cannot afford and this impairs the optimum usefulness of the supplement, for no amount of bonemeal will make up for a general shortage of food that exists.

(6) Difficulty is experienced by farmers in estimating the correct dose. From a practical point of view this is important. The writer once visited a farm in the Vryburg District where well-bred Friesland cows were dying from Lamsiekte, although the farmer was conscientiously feeding bonemeal in what he thought was the correct amount viz. 5 oz. On investigation it was found that he was only giving his cows about 2 oz. of bonemeal instead of 5 oz.

The detrimental effects of excessive handling of stock are very forcibly illustrated by comparing some South African sheep farms. Formerly, on account of the destruction of sheep by jackals and other predatory animals, the sheep had to be collected and kraaled every night. After the enclosing of farms with jackal-proof fencing and the destruction of the jackals, kraaling became unnecessary. Such farms were then able to carry more sheep, which in their turn were healthier and in better condition than on the farms where the old system of kraaling still prevailed. The free grazing thus provided for sheep has contributed very largely to the phenomenal development of the sheep and wool industry of the Union during the last few decades.

Interesting estimates are to be found in the report of the Drought Commission. From information supplied by experienced sheep inspectors in different parts of the Union, it is estimated that those areas, where woolled sheep are usually kept, could carry 54 per cent. more sheep if the animals were given free grazing with a proper system of paddocking. The Commission, furthermore, maintained

that, if these improvements in farming methods could be made, the number of woolled sheep, then estimated at 23,000,000, could be increased to 35,000,000, and that sheep would be healthier, grow better and produce more wool under these improved conditions.

The experience of the last ten years has amply justified these contentions of the Commission. During this time many more farms in the Union have been enclosed with jackal-proof fences, and in other areas the jackal has been so nearly exterminated that free grazing has become possible even without the special fences. After the publication of the Report of the Drought Commission in 1923, the number of woolled sheep owned by European farmers increased from 23,000,000 in 1923 to 34,500,000 in 1928 (1929).

Cattle farmers who are compelled by a phosphorus deficiency to hand feed bonemeal to their stock, are placed under very much the same conditions as the sheep farmer, who, on account of the jackal menace, is forced to collect and kraal his sheep every night.

(c) *Feeding of Bonemeal, etc., as a Lick.*

Here various methods have been advanced:

1. "*Small Box System*".—Theiler, Du Toit and Green (1923) have suggested the use of numerous small feeding boxes. These should be conveniently placed in an enclosure. In each box the requisite amount of bonemeal for an animal is placed. The cattle are then driven in batches into the enclosure, and they are easily trained to go to the boxes to eat the bonemeal. This method eliminates the use of a crush, but the other disadvantages mentioned under "*Handfeeding*" remain, and it has not become popular anywhere. As far as can be ascertained, no farmers have adopted it for any length of time.

2. *Licks given ad lib.*—*The bonemeal is put into large troughs or other containers, and the animals are given free access to the lick.* This is the method adopted on very large farms and cattle ranches where the daily collection of all cattle becomes an impossibility.

The main disadvantages of this system are:

(1) *It is costly.*—Animals will, if given free access to the bonemeal, consume a much larger quantity of bonemeal than is actually necessary. Green (1927) records a case where a cow consumed as much as 4 lb. of bonemeal daily over a prolonged period, when 5 oz. only would have been sufficient. There is reliable evidence from farmers who are using this method that it costs approximately £1 per animal per annum to feed bonemeal in this way. The cost of giving 3 oz., the dose prescribed for dry animals, amounts to 5s. per annum. (This estimate is made reckoning bonemeal at £8 per ton.)

(2) The weaker animals, usually those in the greatest need of phosphorus, are kept away from the bonemeal troughs by the stronger ones, and do not get their fair share of the bonemeal. A considerable amount of scrimmaging takes place round these troughs, and animals are apt to be injured.

(3) A considerable portion of lick is forced out of the troughs by the animals, and this is wasted. It often happens that a trough with its entire contents is upset. Care must be taken to prevent the bonemeal from getting wet. The bonemeal usually used by farmers still contains a small amount of protein (meat), and this wet bonemeal exposed to the hot South African climate affords excellent conditions for the growth of putrefactive and even specific toxin-producing organisms, like the *Clostridium botulinum bovis*. Instances have been noted by the writer where maggots have developed in such bonemeal, which is unfit for animal consumption and may even contain Lamsiekte toxins.

(4) Preparations like di-calcium phosphates are apparently not very palatable to animals, and a lick consisting entirely of these chemicals is not readily taken. To entice animals to take a lick prepared from these materials, salt has to be added. It is known that in many parts of the Union, especially along the coast where there is a marked deficiency of phosphorus, animals refuse to take salt, and in such areas a lick mixed with salt is not taken by them. On the other hand, the optimum amount of salt for an animal is not known definitely, and it is quite possible that where such a lick is taken, the animals may consume an excessive and therefore injurious amount of salt.

(5) Bonemeal is eaten by cattle because they develop osteophagia when grazing on a phosphorus deficient pasturage. Green (1927) found that osteophagia is reduced in sick or semi-starved animals. There is also a natural variation in the degree of osteophagia occurring in different individuals; in some animals osteophagia does not develop to any marked extent, although other symptoms of aphosphorosis may be obvious. It can be understood that under these circumstances there is no assurance that all cattle will consume a bonemeal lick.

(d) *Correcting the P_2O_5 deficiency in the soil with phosphatic fertilizers and in this way improving the P_2O_5 content of the vegetation.*

Top dressing of pastures with artificial fertilizers is extensively carried out in parts of Australia and New Zealand. Investigations are being conducted in various parts of the Union to ascertain the improved grazing condition of the veld when treated with various fertilizers. Results may prove that the carrying capacity and other characteristics may be so improved by using fertilizers that no objections could be raised against the cost. The value of the land in those areas where this extreme deficiency of phosphates is encountered is so low, however, that the cost of adequate fertilization would exceed the present market value of the ground. Moreover, it has yet to be shown that phosphorus fertilizers will raise the P content of the natural vegetation adequately throughout an entire season for the requirements of stock. It must be admitted, however, that pasture fertilization may play a very important rôle in improving the veld.

II. AVAILABILITY OF PHOSPHORUS TO THE ANIMALS IN THOSE MATERIALS COMMONLY USED AS SUPPLEMENTS.

In the paper by Green (1927) on osteophagia and its relation to lambsiekte, the following statement was made: "This question of the 'availability' of phosphorus contained in various substances is of considerable practical consequence, however, since it is generally considered that bonemeal, or even bonedust, is only utilized in digestion to the extent of one-fifth, and a more readily available phosphate should therefore prove cheaper in practice. The whole experiment already carried out suggest that finely ground bonemeal is more readily utilized in digestion than is generally considered to be the case."

Recent investigations by Otto (unpublished data, Dept. Vet. Ser., South Africa), indicate that actually a considerable portion of the phosphorus in bonemeal can be utilized by the animal. Working with two two-year old oxen suffering from a phosphorus deficiency he found that as much as 68 and 70 per cent. of the P_2O_5 in 3 oz. feeding bonemeal was digested and absorbed by the animals.

Further evidence of the amount of the phosphorus which can be assimilated by cattle is provided by the results of a large field experiment planned by Du Toit and Green (1930) to determine the relative value of di-calcium phosphate and bonemeal as sources of phosphorus to cattle. They found that $\frac{2}{3}$ oz. of $CaHPO_4$ (42 per cent. P_2O_5), was very nearly as effective as 3 oz. bonemeal (22 per cent. P_2O_5) and, furthermore, suggested that the latter daily dose probably represented an excessive amount and considered that a smaller dose (2 or $2\frac{1}{2}$ oz.) would probably have given almost the same results as the 3 oz. bonemeal for satisfying the phosphorus requirements of young growing cattle on the phosphorus deficient veld of Bechuanaland. If it is now assumed that all the P in the finely prepared $CaHPO_4$ was made use of by the animals, which was more than likely the case, then a corresponding amount of P was utilized in the bonemeal, which is about 8 gm. P_2O_5 . This quantity represents 64 per cent. of the phosphorus contained in Du Toit and Green's suggested minimum adequate ration of 2 oz. bonemeal, a percentage corresponding very closely to that found by Otto to be actually digested in his tests.

On the assumption that about 60 per cent. of the P in bonemeal is utilized, it is evident that the administration of bonemeal to animals as a source of phosphorus is wasteful, for stock only derive advantage of a little more than half of the mineral contained in it, much of the P contained in the bonemeal passes out undigested with the faeces. It has been held that this undigested phosphorus is not really lost, as it is distributed on the veld and raises the P_2O_5 content of the soil and vegetation. It is, however, doubtful if the phosphorus added to the soil in this way will have any very appreciable effect on the soil and grazing. The carrying capacity of Bechuanaland veld is estimated for cattle at about 5 to 10 morgen per animal. Considering the large area over which cattle range little manure is added to the soil. Thus if 10 morgen of it is required per beast, and if such an animal be given 3 oz. of bonemeal on each weekday and that about 60 per cent. of the phosphorus in this supplement is digested and

utilized by the animal, then during the year only about 1.0 by 300 oz. or about 20 lb. is deposited on 10 morgen of veld. In reality on South African farms a large portion of cattle dung is used as fuel, so that the amount of phosphorus which could be added to the soil in this way is further minimized.

As a direct contrast to this loss and waste the phosphorus balance experiments now being undertaken by Otto show that 100 per cent. of the phosphorus contained in the water soluble phosphates (Na_2HPO_4) can be utilized by the animal.

Bonemeal as a possible source of infectious diseases.

A fact which should not be overlooked is that bonemeal can harbour disease-producing organisms, e.g. Anthrax, Blackquarter, and Tuberculosis, to mention only a few. For this reason all bonemeal sold as a fertilizer or a stock food, is required by law to be certified free of any disease infection. In South Africa as a further precaution, samples of every consignment of bonemeal imported or manufactured in the country are submitted to bacteriological examination. It is of interest to mention in this connection that the Anthrax spores are so resistant that special conditions should be observed in the course of manufacturing di-calcium phosphate from bones in order to destroy any living spores that might be present. Wedemann (1931) recommends that the concentration of the salts in macerating fluid should not be allowed to rise above 10° Bé and the precipitated di-calcium phosphate should be heated three times at 24-hourly intervals at a temperature of $70-75^\circ$ C. to make certain that the calcium phosphate is free from Anthrax infection.

III. SUGGESTED NEW METHOD OF ADMINISTERING PHOSPHORUS TO ANIMALS IN THE DRINKING WATER.

From the foregoing it should be clear that any method which will eliminate some of the disadvantages of the present systems of giving phosphorus supplements to animals will be of tremendous practical value to the farmers. With this in view an attempt was made to develop a method of supplying cattle with the necessary phosphorus in the form of water soluble phosphates and to administer this through the drinking water.

Green (1927) in order to prove that osteophagia was specifically due to a phosphorus deficiency, gave cattle phosphoric acid in their drinking water and also mixed it with maize paste. He found that an amount equivalent to 7 to 9 gm. of P_2O_5 was slow in removing pica, but when the amount was doubled the effect was prompt.

Before describing the actual experimental work, it will be necessary to give a cursory account of the water supply for stock in South Africa, especially in those parts where the phosphorus deficiency is extreme. In very big areas at least 90 per cent. of the water supplied to stock is derived from boreholes or wells, and in some cases from natural fountains. This means that the water supply of stock is easily controlled. The water is usually pumped into reservoirs and then taken by piping to drinking troughs. A water soluble material

could in such cases easily be added to the water in the reservoirs and supplied to the animals. The amount of such a material administered can be controlled by varying the concentration according to the amount of water consumed by the animals in different seasons of the year. No reference could be found to a systematic South African study on the normal amount of water consumed by various classes of stock grazing on the veld. In a country like South Africa, which is so often subjected to long droughts, such a study will be useful.

Having no information about the amount of water consumed by animals and how this amount varies with the state of the grazing, season, size, condition of the animal, etc., observations were made on the water consumption of animals used in this particular experiment to be described further on. The information obtained was immediately applied to control the concentration of the water soluble phosphate added to the drinking water.

EXPERIMENTAL WORK.

The experimental work to be described was undertaken at the Government farm "Armoedsvlakte", Vryburg District, Bechuanaland.

I. ARRANGEMENTS OF WATER SUPPLY OF ANIMALS WHICH WERE GIVEN PHOSPHATES THROUGH THE DRINKING WATER.

A special concrete circular tank with a capacity of 1,700 gallons was erected. The amount of water contained could at any time be determined by means of a fixed scale, having graduations for every 50 gallons. From this supply tank the water was led to a trough which filled automatically, being provided with a ball valve. The arrangement of the trough and tank is well illustrated on the accompanying photograph (Plate I). The water was obtained from a central reservoir of the laboratory, and this reservoir was filled from boreholes. The water supply was situated in a corner of the paddock in which the animals receiving phosphate through the water were grazed.

II. THE PADDOCK.

On account of various difficulties, use had to be made of existing fences on the experimental farm, and a portion of veld approximately 250 morgen in extent was used as grazing for the experimental animals. Facilities were not available for the erection of other camps in close proximity, in which to keep control animals.

III. THE ANIMALS USED AND THEIR GENERAL TREATMENT.

Two classes of animals were used.

Class A.—This consisted of weaned calves, approximately 9 months old. They represented grade Friesland, Afrikaner, Sussex and Red Poll types. A batch of 64 calves was available for a comparative test to ascertain the value of various materials as a source of phosphorus for ruminants. Groups containing 8 individuals, 6 tollies and 2 heifers, were formed. The animals were so distributed

in the various groups that each group contained as nearly as possible the same types of animals. Due regard was given to the weights of the animals when the experiment was commenced, the average weight of the animals in the various groups being very nearly the same, except in the case of the control group, i.e. the group where no supplementary phosphorus ration was given. This group contained heavier and more robust animals, this selection having been purposely made so that no doubt could arise later about the inferiority of the control animals. Of the 8 groups of animals in this comparative phosphate experiment the following will be considered:

Group I.—Received phosphates through the water, and comprised animals—

No.	3038	Tollie
„	3043	„
„	3053	„
„	3064	„
„	3076	„
„	3082	„
„	3069	Heifer
„	3073	„

Group II.—Dosed with bonemeal each day except Sundays. The animals in this group were—

No.	3014	Tollie
„	3015	„
„	3025	„
„	3045	„
„	3061	„
„	3074	„
„	3049	Heifer
„	3057	„

Group III.—Dosed with the same phosphate as Group I. The animals in this Group were:

No.	3026	Tollie
„	3030	„
„	3060	„
„	3067	„
„	3083	„
„	3097	„
„	3059	Heifer
„	3071	„

Group IV.—(Controls (no supplementary phosphate). There were:

No.	3008	Tollie
„	3017	„
„	3028	„
„	3046	„
„	3062	„
„	3080	„
„	3010	Heifer
„	3050	„

Class B.—3-year old oxen. These animals had been used as controls in a previous experiment planned to determine the relative value of di-calcium phosphate and bonemeal. They were also grade Afrikaner, Sussex, Red Poll and Friesland cattle, and were all typical examples of animals suffering from a deficiency of phosphorus.

Two Groups were made :

Group I.—Containing the following and given phosphates in the water :

No.	2269	$\frac{1}{2}$	bred Red Poll ox.
„	2308	$\frac{1}{2}$	bred Red Poll ox.
„	2416	$\frac{1}{2}$	bred Afrikaner ox.
„	2426	$\frac{1}{2}$	bred Sussex ox.
„	2442	$\frac{1}{2}$	bred Sussex ox.
„	2454	$\frac{1}{2}$	bred Friesland ox.
„	2405	$\frac{1}{2}$	bred Friesland ox.

Group II.—Kept as controls :

No.	2265	$\frac{1}{2}$	bred Friesland ox.
„	2384	$\frac{1}{2}$	bred Friesland ox.
„	2384	$\frac{1}{2}$	bred Friesland ox.
„	2356	$\frac{1}{2}$	bred Sussex ox.
„	2427	$\frac{1}{2}$	bred Sussex ox.
„	2397	$\frac{1}{2}$	bred Red Poll ox.

Group I of this class were kept in the same paddock as Group I of Class A. They obtained their phosphates in the same water supply.

Groups II, III and IV of Class A and Group II of Class B were grazed together in different camps on the farm Armoedsvlakte. Whenever the grazing became less plentiful in any camp the animals were put into another camp. They were collected every morning and put through the crush, when those receiving a supplementary phosphorus ration were dosed with that particular phosphate.

The animals receiving the water soluble phosphate through their water supply were grazed together in the special paddock described. They were collected only for weighing, osteophagia (pica) testing and in summer for dipping.

It does not seem as if this arrangement of the grazing allowed to the various animals minimized the significance of the results obtained. The outstanding points under consideration were the phosphorus deficiency as a *limiting factor* in the growth and development of cattle, and methods of correcting this deficiency in the animal.

Green (1927) makes the following statement in connection with the P_2O_5 content of Armoedsvlakte soil: "Reference to the analysis shows that all (soil samples) are characterized by low phosphorus content, the total P_2O_5 ranging from 0.03 to 0.12 per cent., and the available P_2O_5 from 0.0005 per cent. to 0.0022 per cent. The lower figures are more characteristic than the higher figures for the general soil of the farm . . ." It can, furthermore, be stated that the same degree of aphosphorosis will develop in cattle irrespective of the particular camp of natural veld in which they are grazed on the farm Armoedsvlakte.

Theiler, Green and Du Toit (1924) observed that cattle suffering from a deficiency of phosphorus showed decreased consumption of food. Du Toit, Malan and Rossouw (1930) confirmed this in sheep. They found that sheep on a phosphorus-deficient diet consumed progressively less food than animals getting an adequate supply of the mineral. At the end of a feeding test of two years' duration the ewes on the phosphorus-deficient diet consumed an equivalent of only 63 per cent. of the amount eaten by the ewes on a phosphorus-sufficient diet. There can be little doubt that an important cause of the retarded growth of phosphorus-deficient animals is decreased food consumption. A ration (and presumably pasture) which gives excellent growth to sheep if phosphorus is provided regularly, results in poor development and retarded growth in another group of sheep if the phosphorus supplement is omitted. As far as could be ascertained the food was utilized equally well in both groups; the main nutritional difference lay, as already stated, in the quantity of food eaten. On the strength of the aforesaid, it seems safe to assume that, if the control animals had actually grazed on the same camp as those receiving the water soluble phosphates, the differences in their rates of growth would most probably not have been affected.

With the two classes of animals an opportunity was afforded of ascertaining the following:—

1. The practical possibility of administering phosphorus and minerals to animals through their water supply.
2. A comparison of the relative growth, development, etc., of the group receiving the P_2O_5 in the water with those features of—
 - (a) the group receiving the same phosphate by the method of hand-feeding;
 - (b) group receiving bonemeal by method of hand-feeding;
 - (c) group with no P_2O_5 supplement.
3. In the older class of animals, Class B, the efficacy of this method of administering phosphorus through the water could be ascertained in the case of animals suffering from a marked degree of apophorosis.

IV. THE CHOICE AND THE AMOUNT OF PHOSPHORUS CONTAINING SUPPLEMENTS ADMINISTERED TO THE VARIOUS GROUPS.

(a) *The Phosphorus containing Supplements.*

Some water soluble phosphates, notably mono-ammonium phosphate and di-ammonium phosphate are being prepared on a large commercial scale. The fact that the ammonium phosphates are being used as fertilizers indicates that they are obtainable at reasonable prices. Mono-ammonium phosphate containing 56·5 per cent. water soluble P_2O_5 has been quoted at £18. 10s. per ton f.i.b. English ports. Considering the amount of P_2O_5 present in this salt, it does not cost more than bonemeal, which contains approximately 20 per cent. P_2O_5 and is sold at £9 per ton (retail).

It has been clearly proved by Green (1927) that the base in combination with the phosphorus plays an inconsiderable part in the reduction of osteophagia (the term osteophagia being used here as a symptom of aphosphorosis in cattle). Thus calcium phosphate, whether pure or in the form of bonemeal, sodium phosphate and even pure phosphoric acid, are all equally effective in reducing osteophagia. Whether a particular phosphorus-containing substance could be utilized as a supplementary food for removing aphosphorosis depends entirely upon the availability of the phosphorus to the animal. Thus Green (1927) found that the phosphorus contained in ground mineral phosphate (Saldanha Bay rock phosphate) was too refractive to animal digestion and had no effect in the reduction of osteophagia.

The choice of a soluble phosphate for this experiment was not limited to those combined with a particular base. Any of the water soluble phosphates could have been used. When the experiment was commenced, di-sodium phosphate was available in sufficient quantities and this salt was selected. Sodium phosphate has been fed to animals by other workers, and Green (1927) used this salt for preventing osteophagia in cattle; Eckles, Becker and Palmer (1926) found that the subnormal rate of growth in cattle could be rectified by its use.

Du Toit and Green (1930) found that $\frac{2}{3}$ oz. of di-calcium phosphate containing 42 per cent. P_2O_5 was apparently as effective as 3 oz. bonemeal in satisfying the phosphorus requirements of young growing cattle at Armoedsvlakte. From the results of this experiment it was considered that $1\frac{1}{2}$ oz. of Na_2HPO_4 , containing 20 per cent. P_2O_5 , would be equally effective:

$\frac{2}{3}$ oz. di-calcium phosphate contains 0.28 oz. P_2O_5 .
 $1\frac{1}{2}$ oz. di-sodium phosphate contains 0.33 oz. P_2O_5 .

$1\frac{1}{2}$ oz. of this di-sodium phosphate was given daily, except on Sundays, to the animals in Group III of Class A. To ensure that the animals were getting the correct dose, a small tin, which when filled contained the correct amount, was used to dose with. The salt was administered dry, and no difficulty was encountered in getting the animals to swallow it. The same amount of di-sodium phosphate was allowed to those animals to which the salt was being administered through the drinking water. These animals of course received their supplement on Sundays as well. The concentration of the salt in the water was modified according to the amount of water which the animals consumed. Meigs and Woodward (1921) fed amounts up to 1 lb. of crystallized Na_2HPO_4 to cattle without producing any noticeable digestive disturbances whatever. In view of this, it was considered that the much smaller amount ($1\frac{1}{2}$ oz.) fed in this experiment could not possibly set up digestive disturbances.

The bonemeal fed group (Group II, Class A) were given 2 oz. bonemeal (± 22 per cent. P_2O_5) daily except on Sundays. From the results of the experiment of Du Toit and Green (1930), already quoted, it was considered that 3 oz. of similar bonemeal was probably an excess dose, while 1 oz. was too small. For this reason it was

decided to use 2 oz. bonemeal in this experiment instead of the usual 3 oz.* To ensure that the animals were getting the correct amount, the total amount of bonemeal which had to be fed to the group was carefully measured off each day. As it has been found more convenient to give the bonemeal to animals in a wet state, the measured quantity of bonemeal was moistened in a special container and the eight doses were measured off by approximation with a suitable spoon. It cannot be claimed that the individual doses were exactly the same, but over a period the error becomes very small, and it can be considered that over the experimental period these animals received the same amount of bonemeal.

(b) The addition of Sulphuric Acid to the Water for complete Solution of the Phosphate.

When di-sodium phosphate is added to the Armoedsvlakte borehole water containing a large amount of calcium and magnesium salts in solution (120 mg. CaO and 85 mg. MgO per litre) some of the phosphate added is precipitated. It was found that when 45 gm. Na_2HPO_4 (20 per cent. P_2O_5) were added to 6 gallons of the water, about 20 per cent. of the P added was contained in the precipitate. Owing to the excess of hydroxyl ions in the water (pH 8.8) conditions are favourable for the formation of the insoluble calcium and magnesium phosphates, as for example, according to the following equation:



The converse of the precipitation of calcium and magnesium phosphates holds equally good, viz., that the addition of a mineral acid to a solution containing insoluble Ca and Mg phosphates, e.g. CaHPO_4 will favour the formation of the soluble Ca and Mg phosphates $[\text{CaH}_2(\text{PO}_4)_2]$.

To obtain complete solution of the phosphate a small quantity of sulphuric acid (90 per cent. commercial) was added to the drinking water. It was found that 1 c.c. per gallon was sufficient. On account of the more or less constant composition of the mineral salts in the borehole water, it was not necessary to modify the amount of acid to be added in any way during the progress of the experiment.

The following is an analysis of the Armoedsvlakte borehole water used:

1,000 c.c. contains: P_2O_5 —nil, SO_3 —trace, Fe_2O_3 —trace,
MgO—85 mg. CaO—120 mg. Cl—31mg.

* In May, 1930, i.e., after the experiment had been in progress for about six months, the dose of bonemeal was increased to 3 oz. The reasons for making this change are fully explained in the article by du Toit *et al* published in this report.

The following pH. determinations, etc., were made in connection with this water. The pH. value of the untreated water is 8.8. After the addition of 1.6 gm. Na_2HPO_4 (20 per cent. P_2O_5) to 1 litre of water, i.e. an amount corresponding to $1\frac{1}{2}$ oz. per 6 gallons water, the pH. value of the filtered solution was 8.1. To obtain complete solution of the precipitate, it was necessary to add 52 c.c. of N/10 H_2SO_4 to 1 litre of the solution, and the pH. value was 7.0 after the addition of the quantity of acid.

It was found that the mono-sodium and mono-ammonium phosphates were completely soluble in this water, and when a certain amount of these salts was added to correspond to the amount of P in the quantity of di-sodium phosphate added in the above instance, the pH value of the solution in both cases was 6.9.

As 1 c.c. H_2SO_4 (90 per cent.) was added to a gallon of water, and as the animals consumed approximately 6 gallons per day, they received an appreciable amount of sulphate in this way (approximately 12.5 gm. expressed as SO_4). This sulphate is considered to have had no detrimental effect on the animals. Eckles, Becker and Palmer (1926) gave 85 grams of Magnesium sulphate (or 68 gm. SO_4) with a "basal" ration of sodium phosphate to five animals for an entire year, without any harmful effect and no apparent signs of interference with the absorption of the phosphorus.

Small laboratory tests with mono-sodium and mono-ammonium phosphate indicate that in the approximate amount in which they would have to be added to the water in order to administer the required dose of P_2O_5 to cattle, the acid nature of these salts was sufficient to prevent any precipitate from forming. If the mono-basic salts can be used, it will not be necessary to add any additional acid. This is obviously a great advantage, as it will obviate the handling of dangerous acids by farmers who may have no knowledge of their properties.

These mono-basic salts contain less water of crystallization and a much higher amount of phosphorus per unit weight. It seems as if such salts should be used in practice as this more concentrated form of phosphorus would reduce costs of transport, etc.*

* Theiler and Green in reviewing this paper in their article on "Aphosphorosis in Ruminants" (Nutrition Abstracts and Reviews, Vol. 1 No. 3), make the following additional remarks in regard to the use of sodium and ammonium phosphates:—

"According to the views of Meigs and Woodward sodium phosphate increased calcium assimilation and according to the views of Theiler, Green and du Toit a low Ca/P ratio may encourage maximum utilization of calcium, so that the use of acid phosphate, on pastures in which percentage CaO exceeds percentage P_2O_5 , is not contra-indicated. Nor is the use of di-ammonium phosphate contra-indicated on pharmacological grounds since the quantity of ammonium base associated with the prescribed phosphoric acid is small and practically within the reach of bacterial synthesis in the intestine and subsequent utilization as food protein. Indeed in cases in which calcium predominates heavily over phosphorus in the dry matter of the grass the use of ammonium phosphate—an easily metabolizable base associated with a fixed acid—might easily improve absorption of calcium."

(c) Addition of a trace of Copper Sulphate to inhibit the growth of Algae.

The reservoir or mixing tank was not covered in and during the hot summer months the water containing the phosphates in solution offered very suitable conditions for the growth and development of green algae. To stop their growth a small quantity of copper sulphate was added to the water. An amount corresponding to a dilution of 1:250,000 effectively prohibited the growth of the algae. To every 1,200 gallons of water $\frac{3}{4}$ oz. of CuSO_4 was added. The trace of copper sulphate contained in the water had no harmful effect on the animals.

THE RESULTS OF THE EXPERIMENT.

For convenience the results of the experiment will be given and discussed separately under the following headings:—

- I. The Amount of Water Consumed.
- II. The Weight of the Experimental Animals.
- III. Osteophagia in different Groups.
- IV. Clinical Observations:
 - (a) *Styfsiekte.*
 - (b) *Lamsiekte.*
 - (c) *Anaplasmosis.*
- V Inorganic Phosphorus determinations in the blood of the Animals.

I. THE AMOUNT OF WATER CONSUMED BY THE ANIMALS DURING THE PROGRESS OF THE EXPERIMENT.

The water containing the sodium phosphate, sulphuric acid and the trace of copper sulphate was readily taken by the animals. As a matter of fact, it was often observed during the course of the experiment that the animals getting the treated water persistently refused to drink other water. When the experiment was terminated at the end of 1930 difficulty was encountered in getting these animals to drink the ordinary water on the farm, and they had to be retained in the paddock with the special tank and trough and given ordinary water for some time to accustom them to drink it again. The liking of the animals for this treated water is an obvious advantage in this system of administering phosphorus, because when once accustomed to this water animals will avoid drinking any other water which might be accessible to them.

As already mentioned, no reliable data about the amount of water consumed by animals under South African veld conditions were available. Before the experiment was commenced, sixty nine-months old weaned calves were kept in the paddock in which the special tank has been erected. Daily observations of the amount of water consumed were made. These observations are given in Table I.

TABLE NO. 1.

THE AVERAGE AMOUNT OF WATER CONSUMED BY A GROUP OF NINE-MONTHS OLD WEANED CALVES.

Date: November, 1929.	5th.	6th.	7th.	8th.	9th.	10th.
Total amount of water in tank at 9 a.m.....gallons	1,700	1,425	1,200	950	725	525
Total amount consumed in 24 hours.....gallons	—	275	225	250	225	200
Number of calves.....	60	60	60	60	60	60
Average amount of water per animal (daily).....gallons	—	4.58	3.75	4.17	3.75	3.33

Daily individual average over this period: 4.16 gallons.

Over this period of five days it was found that an average amount of 4.16 gallons was consumed by each animal, and this information was used to determine the amount of sodium phosphate which had to be added to the first tankful of water in order to allow an animal to get approximately 1.5 oz. of di-sodium phosphate per day.

As already mentioned, 15 animals were given the phosphate containing water. These consisted of 8 animals of Class A (weaned calves) and 7 of Class B (three-year old control oxen). Although there was a considerable difference in the age of these animals, there was no great difference in their weight. The animals of Class A averaged 366 lb., and those of Class B, 539 lb. On account of this relatively small difference it was considered that the two classes of animal would consume approximately the same volume of water. This proved actually to be the case. Observations on the amount of water consumed by these 15 animals were made and the results are given in Table No. 2.

TABLE NO. 2.

Readings made at 9 a.m. on:	12.11.29.	19.11.29.	21.11.29.	27.11.29.
No. of days.....	—	7 days	—	6 days
Total volume of water in the tank	1,725 gals.	1,125 gals.	875 gals.	450 gals.
Total volume of water consumed	—	500 gals.	—	425 gals.
Number of cattle.....	15	15	15	15
Average amount of water per animal per day.....	—	4.76 gals.	—	4.72 gals.

The 15 animals consumed over this period an average of 4.74 gallons of water per day. After this no observations were made at short intervals on the amount of water consumed. The tank was filled to a convenient level, generally 1,200 gallons, and when this water was finished the average amount consumed by the animals over that period was calculated.

The volume of water consumed and the amount of sodium phosphate sulphuric acid and copper sulphate added to the water will be found detailed in Table No. 3.

TABLE NO. 3.

RECORD OF WATER CONSUMPTION, SODIUM PHOSPHATE AND H_2SO_4
USED BY ANIMALS IN EXPERIMENT.

Date.	Amount of Water Added to Tank.	No. of Animals.	Average Amount of Water Consumed per Animal.	Weight of Na_2PHO_4 Added.	Volume H_2SO_4 Added.	Weight CuSO_4 Added.
	Gals.		Gals.	lb.	c.c.	Oz.
1.11.29.....	1,000	15	4.75	20	1,000	—
27.11.29.....	1,000	15	4.72	20	1,000	—
13.12.29.....	1,500	15	5.0	28	1,500	—
2.12.29.....	1,500	15	5.0	28	1,500	—
17.1.30.....	1,200	15	6.0	19	1,200	—
2.2.30.....	1,200	15	6.0	19	1,200	1
12.2.30.....	1,200	15	6.0	19	1,200	.75
26.2.30.....	1,200	15	6.0	19	1,200	.75
10.3.30.....	1,200	15	6.0	19	1,200	.75
24.3.30.....	1,200	15	6.0	19	1,200	.75
12.4.30.....	1,200	15	4.0	28	1,200	.75
28.4.30.....	1,200	14	4.2	26.5	1,200	.75
16.5.30.....	1,200	14	5.0	22.5	1,200	.75
2.6.30.....	1,200	13	4.3	26	1,200	.75
23.6.30.....	1,200	13	4.4	25.5	1,200	.75
14.7.30.....	1,200	13	4.6	24.5	1,200	.75
4.8.30.....	1,200	13	4.6	25	1,200	.75
25.8.30.....	1,200	13	4.6	25	1,200	.75
15.9.30.....	1,200	13	4.5	25	1,200	.75
3.10.30.....	1,200	11	6.1	18.5	1,200	.75
25.10.30.....	1,200	10	5.5	20.5	1,200	.75
8.11.30.....	1,200	10	8.57	13	1,200	.75
22.11.30.....	1,200	10	8.0	14	1,200	.75
6.12.30.....	1,200	10	8.0	14	1,200	.75
20.12.30.....	1,200	10	8.5	13	1,200	.75

Total number of days of experiment: 411.

Number of days \times number of animals: 4,417.

Total amount of Na_2HPO_4 consumed: 431 lb.

Average amount each animal received per day: 1.56 oz.

Average amount of water consumed by each animal per day: 5.8 gals.

It will be noticed that the concentration of sodium phosphate was modified from time to time, depending upon the amount of water consumed by the animals during different periods of the year. The average daily water consumption at different times of the experimental period was determined by observing how long each tankful of water lasted the group of animals. Usually 1,200 gallons of water were added on each occasion.

From the information on Table No. 3 it was found that at the end of the experimental period the animals had each on an average consumed 5.8 gallons water per diem. The amount of sodium phosphate allowed to these animals was controlled to a remarkable degree of accuracy. It was found that during this period of over a year each animal had received on average 1.56 oz. of sodium phosphate per day.

The average daily amount of water consumed by these animals during different months of the year is illustrated on Figure 1:

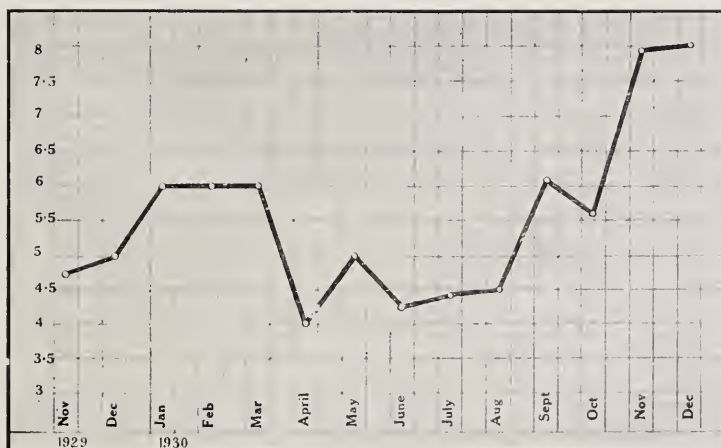


Fig. 1.—The average amount (in gallons) of water consumed by the experimental animals in different times of the year.

It will be noticed that in November, 1929, the animals took approximately $4\frac{1}{2}$ gallons of water daily. This amount increased to 6 gallons during January, February and March (1930). These are the hottest months of the year. The amount then decreased to $4\frac{1}{2}$ gallons, and continued at that level through the winter months. During the next spring and summer the amount increased, and in November and December, 8 gallons were taken every day. The greater amount taken during these months as compared with the same months of the previous year, can be ascribed to the increased size of the animals. The animals now weighed over 700 lb. in Class A and over 800 lb. in Class B, as compared with 400 lb. and 600 lb. respectively in the previous period.

Discussion.

The amount of water consumed by an animal is influenced by some of the following factors:—

- (a) The temperature and humidity of the surrounding atmosphere.
- (b) The size of the animal.
- (c) The water content of the food.
- (d) The state and condition of the animal, e.g. whether cows are lactating or not.

The following statement is taken from Feeds and Feeding by Henry and Morrison (1922): "The amount of water they (milk cows) will drink depends upon the yield of milk, and also on the amount of water in their feed and on the air temperature. Cows in milk require on an average $12\frac{1}{2}$ gallons of water daily, and high producing cows even more. Eckles found that cows in milk drank 4 times as much water as when they were dry and farrow. In one instance a Holstein cow producing 100 lb. of milk a day on a ration of 18 lb. Alfalfa hay, 10 lb. corn silage and 14 to 20 lb. of concentrates, drank from 216 to 307 lb. of water daily . . ."

If a farmer adopts this method of administering phosphates or other minerals to his livestock he can either proceed along the lines on which this experiment was conducted, i.e. alter the amount of soluble phosphates to be added to the amount of water consumed by that particular herd of cattle, and in order to do this he will have to keep a record of the amount of water his animals consume from time to time, or (what seems a much more practical scheme) he can make up a "standardized concentration of phosphates in the drinking water." Obviously this amount will be based upon the relative quantities of water consumed by different classes of stock. From the observations made in this experiment it seems as if 6 gallons of water could be used as a convenient "standard quantity" for *dry mature cattle* and for *young weaned calves* 4 gallons. To these amounts of water a quantity of soluble phosphate equivalent to 10 gm. P_2O_5 should be added.* Let us consider what would happen if these "standardized concentrations of phosphates" in the drinking water were allowed to a herd of cattle.

* From a consideration of the minimum amounts of phosphorus supplements which in practice have been found sufficient for cattle grazing on a phosphorus deficient veld as typified by Armoedsvlakte grazing, the 10 grams P_2O_5 suggested is arrived at thus:

- (1) It is suggested by Du Toit and Green that 2 oz. bonemeal, containing about 12 grams P_2O_5 would probably suffice the requirements of young growing cattle for normal growth and development. Moreover, it has been argued in this paper that apparently about 65 per cent. of the phosphorus in this amount is utilised by the animal, i.e. approximately 8 grams P_2O_5 .
- (2) Two-thirds of an ounce $CaHPO_4$ containing 8 grams P_2O_4 was found to be an effective ration, and apparently all the phosphorus in this material can be made use of.
- (3) Finally the results of this experiment indicate that $1\frac{1}{2}$ oz. Na_2HPO_4 containing 9 grams P_2O_5 is also a sufficient supplement.

1. Small mature animals would get a smaller amount of phosphates as they drank this water. This would be an advantage, as it would mean an improved method of regulating the amount of phosphorus administered to animals of different sizes. Weaned calves and other immature animals require a larger amount of phosphorus in proportion to their size than older animals, and it has therefore been suggested that a more concentrated solution of phosphorus be provided for such animals, viz. 10 gm. P_2O_5 in 4 gallons of water.

2. Lactating animals would get more phosphorus. Milk contains approximately 87 per cent. water, and naturally a larger amount of water is lost by the animal in this way. Individuals with a high milk yield drink more water than others supplying less milk. That an increased supply of phosphorus is necessary for milk cows is obvious and they would regulate this themselves. The amount of $12\frac{1}{2}$ gall. given by Henry and Morrison and just referred to may be used for a further consideration of the amount of phosphorus taken by cows if the supplement is given in the drinking water. If cows are allowed the "standard concentration" suggested for mature dry cattle they would get on an average 20 gm. P_2O_5 per day. This, considering the amount of phosphorus secreted in the milk, and discussed further on, is probably insufficient. It is suggested that such animals be given the "standard concentration" for young animals, i.e. 10 gm. P_2O_5 in 4 gallons of water. In this case the cows would be getting approximately 30 gm. P_2O_5 supplement per diem. How does this compare with the amount of bonemeal usually prescribed to South African farmers? 5 oz. bonemeal containing ± 22 per cent. P_2O_5 is generally used; the animals in this case get $\pm 31\text{--}35$ gm. P_2O_5 , all of which is not available to the animal. In the case of the soluble phosphate all the phosphate contained in the water can be utilized by the animal. In a recent review by Crichton (1930) on the mineral requirements of cattle, it is stated that persistently negative balances have been found in the case of calcium, phosphorus, magnesium, etc. (in the lactating cow), and therefore it will be interesting to determine to what extent this negative balance of phosphorus could be reduced by this method of administering phosphates to lactating cows, especially in Bechuanaland, where there is an abundant supply of calcium, not only in the vegetation but also in the water.

It may be considered that 2 gallons of milk per day is an average yield of veld-fed and bonemeal-dosed cows in Bechuanaland. On the assumption that milk contains 120 mg. P per 100 c.c., such an animal loses about 25 gm. P_2O_5 through the milk every day. It is obvious that this depletion of P_2O_5 from the animals could be more readily reduced by the 30 gm. easily available P_2O_5 in the postulated $12\frac{1}{2}$ gallons of drinking water than the corresponding amount indifferently assimilable P_2O_5 in the 5 oz. bonemeal. The use of soluble phosphates for animals has been favoured by other authors. Meigs and Woodward (1921) suggest that the assimilation of phosphorus by pregnant cows, and probably that of calcium also, is favoured by adding *disodium phosphate* to the feed.

3. *More phosphates will be taken during the summer than in winter* on account of the greater amount of water taken by the animals during the hot months. In the summer rainfall areas of the Union

the grazing is very dry in the winter and generally green in summer. This increased water content of the grazing in the summer naturally tends to reduce the amount of water that animals drink. The difference in the amount of water consumed by animals during these cold and hot periods of the year is not so great as would have been the case if the water content of the grazing remained the same.

On considering the growth and development of veld-fed cattle in the summer rainfall area of South Africa, it is found that in winter, on account of the poor grazing, animals cease to put on weight. Du Toit and Bisschop (1929) have pointed out that this loss of condition during the winter months is considerably reduced, but not entirely overcome, by giving phosphorus supplements. Other factors, e.g. low protein content of the grazing, limit growth and development. Under these circumstances it may be considered that during the winter months the animal requires an amount of phosphorus sufficient for maintenance only. In summer, however, when the grazing is green and plentiful, they rapidly improve in condition and grow at a maximum rate. There is a tendency for farmers to arrange the breeding season of their cows so that the calves are dropped at the beginning of summer.

The phosphorus content of the vegetation has been extensively studied, especially in the phosphorus deficient areas of South Africa, by Green (1927), Staples and Taylor (1929), Heurici (1928). It is generally found that the young grass, usually in October and November after the first rains have fallen, contains a fairly high percentage of phosphorus, but this rapidly falls as the grass matures. The nutritive value of the vegetation, apart from the phosphorus content, does not change as rapidly. To illustrate this point the following table (Table No. 4) is taken from "Phosphorus in the Live-stock Industry" by Theiler, Green, and Du Toit.

TABLE NO. 4.
PROXIMATE ANALYSES OF DRY MATTER OF ARMOEDSVLAKTE
MIXED GRASSES.

Date.	Crude Protein.	Ether Ex- tract.	N-free Ex- tract- ives.	Crude Fibre.	Ash.	P ₂ O ₅ .	CaO.	Estimated Energy Value Starch— 100.	Ration of Starch Equivalent to P ₂ O ₅ .
	° %	° %	° %	° %	° %	° %	° %		
10.11.19...	19.4	5.5	41.0	22.5	11.6	.60	.31	56	100 : 1.07
8.12.19...	14.3	5.6	46.8	25.6	7.7	.32	.59	—	—
15.1.20...	13.8	5.5	48.0	25.0	7.7	.22	.50	52	100 : 0.42
4.3.20...	7.2	3.4	49.8	33.7	5.9	.24	.43	—	—
19.4.20...	4.9	2.4	51.6	35.0	6.1	.11	.46	32	100 : 0.33
11.5.20...	4.1	2.2	52.9	34.9	5.9	.07	.50	—	—
8.6.20...	4.0	2.0	53.7	33.1	7.2	.09	.59	25	100 : 0.36
	European figures for comparison.								
Rich pasture grass.....	20.5	4.6	45.9	19.0	10.0	.7	.9	60	100 : 1.16
Poor meadow hay.....	8.7	1.7	44.8	39.0	5.8	.4	.9	22	100 : 1.81

It will be noticed that the P_2O_5 content of the grass rapidly falls from 60 per cent. in November to 32 per cent. in December, the fall continues and is lowest in the winter months.

To meet the development and growth exhibited by the animals during the summer months, a greater total amount of phosphorus is required by the animals than during the quiescent winter months. This increased P_2O_5 is only partially supplied by the vegetation, and an even greater P_2O_5 supplement than in winter is indicated during the summer when the animals as the result of better grazing show greatest activity in growth and development. From this point of view the larger amount of P_2O_5 consumed by animals on the hypothetically standardized concentration of phosphates in the drinking water is warranted.

II. THE WEIGHT OF THE ANIMALS IN THE DIFFERENT GROUPS.

The animals were weighed every fortnight. Care was taken to allow them to drink their fill before the weighings were made.

The results of the weighings indicate the development and condition of the animals in the various groups.

Class A (two-month old weaned calves).—The weight curves of the groups in this class are given on Fig. 2. It will be noticed that the group getting the di-sodium phosphate through the water showed a more progressively rapid gain than any other group. In Table No. 5 the average weights of the groups at the beginning and termination of the experiment are given:

TABLE NO. 5.

CLASS A.

Group No.	Initial Average Weight, 31.10.29.	Average Weight Termination of Experiment, 28.12.30.	Difference.
I. Na_2HPO_4 in drinking water...	lb. 366	lb. 768	lb. 402
II. Bonemeal dosed.....	362	694	332
III. Na_2HPO_4 dosed.....	354	651	297
IV. No P_2O_5 supplement (controls)	429	545	116

The group getting the Na_2HPO_4 in the drinking water showed a total gain of 402 lb. over this period compared with 332 lb. in Group II, 297 lb. in Group III, and 116 lb. where no P_2O_5 supplement was given.

The average weights of Group I were affected by an outbreak of Anaplasmosis. Two animals No. 3043 and 3082 died from this disease and No. 3053 had a severe attack but recovered. Anaplasmosis is a debilitating disease, and animals suffering from it rapidly lose condition. This was reflected in the weighings. The sick animals reduced the average of the weights obtained. If it had not been for the outbreak of this disease amongst the animals in this group, the weight curve would have been even more striking.

Class B (three-year old oxen).—The weight curves of the groups in this class are given in Fig. 3. As already mentioned, these animals were all typical cases of cattle suffering from a deficiency of phosphorus. They were stunted, low in condition, etc. When the experiment was commenced these three-year old oxen weighed on an average only 600 lb.

It will be noticed that those animals receiving the P_2O_5 in the water gained rapidly in weight and soon passed the control group, which were considerably heavier (112 lb.) at the commencement of the experiment. This gain was consistent, but here again anaplasmosis interfered with the average weight of the group getting the phosphate in the water. Nos. 2426 and 2442 succumbed to the disease, and Nos. 2454 and 2405 had a severe attack but recovered. The average weights of the groups at the commencement and termination of the experiment are given in Table No. 6.

TABLE NO. 6.

CLASS B.

Group.	Initial Average Weight, 31.10.29.	Weight at Termination of Experiment, 28.12.30.	Difference.
I. Na_2HPO_4 in drinking water...	lb. 536	lb. 876	lb. 340
II. No P_2O_5 supplement (controls)	651	769	118

Discussion.

In Class B the animals were already almost mature (3 years), and the increase in weight in Group I must be largely considered as an indication of greater improvement in the condition of these animals compared with the controls.

In Class A the higher rate of gain of the Group I indicates that these animals developed at least as fast as those dosed with bonemeal and were always in better condition. This could easily be seen when the animals in the two groups were judged and compared.

In order to show that the increase in weight of the animals in the experiment under discussion is similar to that in previous experiments, it will be well to glance at the results given in the following table (Table No. 7), compiled from the work of Du Toit and Green, to ascertain the relative value of di-calcium phosphate and bonemeal as a source of P_2O_5 for animals. The materials were dosed to the animals. The animals in this experiment were of the same type, age, etc., as in the experiment under discussion.

TABLE NO. 7.

DI-CALCIUM PHOSPHATE AND BONE MEAL COMPARATIVE TEST.

Group No.	Weight at Commencement of Experiment, 19.10.27.	Weight at 28.12.28.	Difference.
I. 3 oz. Bonemeal per diem.....	lb. 441	lb. 784	lb. 343
II. 1 oz. Bonemeal per diem....	439	719	280
III. $\frac{1}{2}$ oz. Bonemeal per diem....	439	673	234
IV. 2 oz. $CaHPO_4$ per diem.....	439	748	309
V. $\frac{2}{3}$ oz. $CaHPO_4$	440	744	304
VI. $\frac{1}{3}$ oz. $CaHPO_4$	437	724	287
VII. Controls (no P_2O_5 supplement)	429	552	123

It will be noticed that Groups I (3 oz. bonemeal), IV (2 oz. $CaHPO_4$), and V ($\frac{2}{3}$ oz. $CaHPO_4$) gained 342, 309, and 304 lb. respectively. In these cases it was considered that the animals had received a sufficient P_2O_5 supplement (Du Toit and Green, 1930), and the rate of gain was more or less the same as in the bonemeal and Na_2HPO_4 dosed animals in the present experiment. The controls in the two experiments behaved in practically the same way (gained 123 lb. and 116 lb. respectively), over these two separate years and where an adequate phosphorus supplement was administered by the hand-feeding method the animals responded in much the same way in the two different seasons.

The greater gain of the group of weaned calves which obtained their phosphorus through the drinking water must be largely ascribed to the following factors:—

- (a) They were subjected to a minimum amount of handling and interference. Katabolism was reduced and the animals had greater freedom for grazing. This is considered the most important factor.
- (b) The phosphorus was given in an ideal way for absorption, as being altogether soluble in water it mixed intimately with the ingesta, affording a maximum degree of absorption. In the summer the animals received their phosphorus supplement twice daily, as they drank water early in the morning and again towards evening.
- (c) *Differences in Grazing.*—As was mentioned under the heading “The animals and their general treatment”, it was considered that the system of grazing allowed to the different groups of animals would not seriously interfere with the results obtained. It was pointed out that the degree of deficiency of phosphorus was more or less uniform on Armoedsvlakte. This deficiency of phosphorus is the outstanding limiting factor in the growth and development of cattle in this area. It can be said without hesitation that aphosphorosis in cattle will develop even if the animals are allowed unlimited grazing on this farm. As a matter of fact, should one animal be kept on Armoedsvlakte and allowed all the available natural grazing, it would still suffer from a phosphorus deficiency, for the limiting factor in growth is not the amount of available food but its low phosphorus content. This phosphorus deficiency is the outstanding factor under investigation in this experiment, and it was present in the same degree in the grazing allowed to the different groups of animals. It would, however, have been more satisfactory if the amount of grazing allowed to the animals in the various groups had been identical, so that any differences in this respect could have been excluded, but this was difficult to arrange and really not necessary, as the experiment was essentially of a preliminary nature and part of a larger experiment to determine the value of various phosphates including those discussed in this paper.

From a comparison of the weight increase of similar animals in the experiment of Du Toit and Green in a previous year, it seems as if control animals, i.e. where no P supplement is given, have a rate of development which is more or less constant even in different years, and that animals given an adequate phosphorus supplement by the hand-feeding method show a similar constancy in rate of development. In other words, the phosphorus deficiency factor in the case of control animals operates to the same extent in different seasons, and when the phosphorus deficiency is removed, the feeding value of the veld in other respects is more or less similar in different years.

Although difficult to determine, it can be considered that with the number of cattle generally kept on Armoedsvlakte (one beast on 12 morgen), the carrying capacity of the farm is not taxed to its utmost, and, as a matter of fact, the grazing available with a moderate rainfall is really excessive for the number of cattle kept on the farm. In 1929-30 (when this experiment was in progress), good rains fell and the grazing was good (*vide* appendix rainfall records for these years), so that although the animals in the "water groups" had each 16 morgen of veld available and the animals in the other groups had approximately 12 morgen each, the amount of grazing allowed was even excessive in the latter case, especially as they were moved from one camp to another whenever it seemed necessary.

The greater increase in the weight of the group of animals which received their phosphorus supplement in the drinking water could not have been materially influenced by the greater amount of grazing allowed to them. The fact that these animals were seldom handled must be held chiefly responsible for their improved condition when compared with the animals receiving phosphorus supplements by the hand-feeding method. The bonemeal dosed animals of Class A showed a greater gain than those dosed with di-sodium phosphate. It will be noticed in the next chapter that the osteophagia in the group was bad. It seems that the amount of P (9 oz. Na_2HPO_4 per week) was insufficient when compared with the effect of 12 oz. bonemeal; a slightly larger dose would probably have been more effective. This result seems to indicate that a larger proportion of the P in bonemeal can be utilized by the animal than is generally considered and indirectly bear out the findings of Otto already referred to.

III. OSTEOPHAGIA IN THE DIFFERENT GROUPS.

It is now well known that osteophagia or bone-eating is a factor involved in the cause of Lamsiekte. It has been proved beyond dispute by Theiler and his co-workers (1927) that osteophagia is brought about by a deficiency of phosphorus in the animal, and it can be effectively prevented by administering a supplementary phosphorus ration. Theiler and his co-workers devised a system of establishing the degree of osteophagia present in an animal by means of the so-called pica tests. These tests were carried out in the same way as described by these authors (1927), and were made every fortnight on the animals in this experiment. It is possible to distinguish, "Rotten bone cravers", i.e. animals exhibiting a marked degree of craving and usually those that might eat toxic carcass material and contract Lamsiekte, "Sweet bone cravers" where less craving is present, "Lickers" where only a slight degree of craving exists, and "Non-cravers" where craving is absent.

The degree of craving for *rotten bones* exhibited by the animals in the various groups is graphically illustrated on Figs. 4 and 5. These graphs have been made by calculating the percentage number of rotten bone cravers in each group on each pica-testing day.

In Class B (Fig. 4) it will be noticed that the craving for rotten bones gradually diminished, and ultimately was very slight or nil. This is further brought out in the analysis of the pica tests as given in Table No. 8.

TABLE NO. 8.
PICA TESTS IN CLASS B.

Group.	Total Number of Pica Tests.	Percentage Rotten Bone Cravers.	Percentage Sweet Bone Cravers.	Percentage Lickers.	Percentage Negative.
I. Na_2HPO_4 in water.....	196	34.8	22.3	2.1	38.8
II. Controls (No P_2O_5 supplement)	157	97.4	1.3	0	1.3

Discussion.

All the animals (100 per cent.) in both groups showed craving for rotten bones when the experiment was commenced. This craving persisted in the control group but lessened gradually in the groups receiving P_2O_5 in the water, and ultimately fell to nil. Theiler, etc. (1927), found that in rotten bone cravers the craving is remedied only after some time by bonemeal and other phosphorus containing substances. The interval required to reduce craving to a minimum depends largely upon the amount of P_2O_5 administered, but even with excessive amounts the craving only ceases after about six weeks. Green (1927) found that 4 oz. of di-sodium phosphate is as effective as 5 oz. of bonemeal in stopping craving. There is no doubt that if a sufficiently large amount of Na_2HPO_4 had been added to the water the craving would have been reduced to a more satisfactory basis in a much shorter period. The efficiency of even the small amount of P_2O_5 used in the water to stop the craving is readily seen from the information contained in Table No. 9.

TABLE NO. 9.
FURTHER ANALYSIS OF PICA TESTS IN CLASS B.

Group No.	Period.	Total Number of Pica Tests.	Percentage Rotten Bone Cravers.	Percentage Sweet Bone Cravers.	Percentage Lickers.	Percentage Negative (No Craving).
I.	11.11.29- 19.5.30	96	58.3	19.8	3.1	18.8
II.	11.11.29- 19.5.30	77	94.8	4	5.2	0
I.	19.5.30- 29.12.30	88	9.1	35.2	0	55.7
II.	19.5.30- 29.12.30	80	97.5	0	0	2.5

Here the pica tests made during the progress of the experiment have been divided over two equal periods. Any seasonable effect on the pica is excluded, as an equal portion of the winter and summer period is included in the two intervals. It will be noticed that in Group I, craving for rotten bones practically ceased in the second half of the experimental period. The satisfactory "phosphorus equilibrium" to prevent craving was definitely established in this group, although it took rather long, largely as a result of the comparatively small amount of P_2O_5 administered.

In Class A.—The degree of craving shown in the various groups is illustrated on Fig. 5, and an analysis of the pica tests is given in the following table (Table No. 10).

TABLE NO. 10.
ANALYSIS OF PICA TESTS IN CLASS A.

Group.	Total Number of Tests.	Percentage Number of Rotten Bone Cravers.	Percentage Number of Sweet Bone Cravers.	Percentage Number of Lickers.	Percentage Negative. No Craving.
I. Na_2HPO_4 in water.....	223	22.0	17.5	0	60.5
II. Bonemeal dosed	231	9.9	26.0	0	64.1
III. Na_2PHO_4 dosed	231	47.2	15.1	0.8	36.9
IV. Controls. No P_2O_5 supplement	184	64.0	20.2	0	15.8

Discussion.

The craving in Group II (bonemeal fed) is slightly less than in Group I, while in Group III, the craving is bad, but better than in the control group. As already stated, the animals in Group I received a larger amount of di-sodium phosphate than the animals in Group III. Actually Group III received $\frac{1.5 \text{ by } 6 \text{ oz.}}{7}$ per day (not being dosed on Sundays), i.e. 1.29 oz., whereas Group I were given on an average 1.56 oz. or 0.27 oz. more per diem. It seems that 1.29 oz. is just below the minimum amount of Na_2HPO_4 (20 per cent. P_2O_5) necessary to prevent "pica". As has been mentioned, Green had to administer 4 oz. of this salt to eliminate pica, but this was done with a view to reducing pica as soon as possible in order to prevent Lam-siekte in a herd of cattle where osteophagia was bad (i.e. marked aphosphorosis). Subsequent experiments, especially those of Du Toit and Green (1929), indicate that where animals are given their phosphorus supplement from the time that they are weaned, their only food then being the veld, a much smaller quantity of phosphorus is necessary to avoid craving. They found that $\frac{2}{3}$ oz. of di-calcium

phosphate was as effective as 3 oz. bonemeal in this respect. It seems safe to assume that if the animals in Group III were actually given their 1.5 oz. of Na_2HPO_4 as was the case in Group I, pica would have been reduced to a satisfactory basis.

These pica tests, although very useful, are of an artificial nature. Cases have been observed where animals persistently take bones in these tests and have been described by Du Toit and Bisschop (1930) as "chronic cravers", while other animals, although showing other symptoms of aphosphorosis never develop craving, as indicated by these tests. The crucial test for the presence of osteophagia as an indirect cause of Lamsiekte in cattle is the actual appearance of the disease, that is to say, where the craving has been so bad that the animal actually consumed carcass material on the veld. Further reference to this aspect of the results of this experiment will be found under the next heading.

IV. CLINICAL OBSERVATIONS.

(a) *Styfsiekte*.—Theiler, Green and Du Toit (1927) succeeded in definitely establishing that this disease is caused by one factor only, viz. a *deficiency of phosphorus in the animal*. This being the case, the disease disappears and is prevented when the deficiency of phosphorus in the diet is corrected. It is well known that the feeding of bonemeal cures and prevents the disease. This was first observed by Hutcheon (1884) nearly 50 years ago.

When this experiment was commenced five animals in Group I of Class B showed marked clinical manifestations of the disease. These were Nos. 2269, 2405, 2442, 2426 and 2454. They were stiff, and the joints, especially the carpus and meta-carpus, markedly enlarged. All the animals in this class were in very poor condition. Styfsiekte is an insidious disease, and from the description given by Theiler, etc., in the paper just referred to, various degrees of the disease may be distinguished. The animals mentioned as suffering from Styfsiekte were those which were actually stiff and lame. Strictly speaking, all the animals in this class were suffering from the disease, but in the other cases it had not progressed so far as to interfere with the gait. The Styfsiekte in the cases mentioned disappeared after the animals were given the water containing phosphates and did not recur in any of the animals while they received this treatment. Needless to say, Styfsiekte never made its appearance in any of the animals in Group I of Class A, which were also getting the phosphatized water.

It can therefore be definitely stated that the P_2O_5 administered in the drinking water to these animals effectively cured bad cases of Styfsiekte and prevented the reappearance of the disease.

(b) *Lamsiekte*.—Lamsiekte in cattle is caused by the ingestion of carcass debris containing toxins produced by the *Clostridium botulinum boris*. Theiler and his co-workers (1927) very clearly established all the factors involved in the causation of this disease. The deficiency of P_2O_5 in the animal leads to the appearance of osteophagia and allotriophagia. Bonemeal in the first instance was

given to cattle to reduce the osteophagia, and in this way the animals were prevented from consuming any toxic carcass material. The effectiveness of the administration of bone meal as a preventive of this disease is very well known. The osteophagia (pica) tests already referred to in this article, were devised by Theiler, etc., to ascertain the degree of osteophagia present in animals. As these tests are of an artificial nature, it is not absolutely safe to infer from them that animals will not contract Lamsiekte. The actual natural appearance of the disease in a group of animals receiving a supplementary P_2O_5 ration in some form or other seems to be the crucial test of the efficacy of that supplement in preventing that disease. During the progress of the experiment no cases of Lamsiekte occurred in Groups I, II and III of Class A, and in Group I of Class B, while amongst the controls *one* fatal case (2384) occurred in Group II of Class B, and *three* fatal cases (Nos. 3010, 3017 and 3062) occurred in Group IV of Class A.

It has become customary to clear the veld of the experimental farm of any carcass material that may be present. Once a year a systematic search is made and all such material removed when discovered. This search was also made in 1929, but special care was taken not to search the camp in which the animals getting their P_2O_5 in the water were kept. Carcass material was present in this camp. It was near the homestead, and dogs belonging to the officials often killed meercats and hares in the camp. On several occasions such dead animals were found, but they were not removed.

In this way the animals grazing in this paddock ran a greater risk of contracting Lamsiekte from eating toxic carcass material than the animals in the other groups, yet the disease did not make its appearance amongst them. It can therefore be concluded that amongst these animals, over the experimental period, the administration of sodium phosphate in that particular amount through the drinking water, prevented Lamsiekte.

(c) *Anaplasmosis*.—The occurrence of anaplasmosis in the groups drinking the treated water has already been referred to. The disease bears no relation to the conditions of aphosphorosis which were under consideration in this experiment, and no further remarks about it need be made.

(d) As was expected no digestive disturbances could be noticed in any of the groups getting Na_2HPO_4 as a phosphorus supplement either in the drinking water or dosed.

(For particulars of deaths, dates, etc., see appendix.)

V. INORGANIC PHOSPHORUS DETERMINATION IN THE BLOOD OF THE EXPERIMENTAL ANIMALS.

Previous work by Theiler, Green and Du Toit (1927), Malan (1930), and Rossouw (1930) has indicated the value of determining the inorganic phosphorus fraction in blood as a method of diagnosing a phosphorus deficiency in ruminants. Five animals in each group were bled every month and the inorganic phosphorus determined, according to the method described by Green (1928).

The results of these monthly analyses are given in Table No. 11. Only the average figure for the five animals is given. These results are graphically illustrated on Figs. 6 and 7.

TABLE NO. 11.
INORGANIC PHOSPHORUS IN MG. PER 100 BLOOD.

	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	Aug.	Sept.	Oct.	Nov.	Dec.
CLASS A. GROUP I. Na ₂ HPO ₄ in water	2.9	6.4	6.7	5.2	4.6	4.6	4.0	5.6	5.6	5.6	5.9	6.7	4.6
GROUP II. Bonemeal.....	3.1	5.9	5.3	5.3	5.5	4.3	5.4	5.9	6.1	5.6	5.5	6.9	6.0
GROUP III. Na ₂ HPO ₄ dosed....	3.1	4.7	4.5	4.3	5.1	3.6	4.6	5.4	5.9	5.4	5.4	6.8	5.5
GROUP IV. No P ₂ O ₅ supplement	3.2	3.7	3.1	2.8	2.8	2.8	2.5	3.3	3.0	2.6	4.6	3.8	2.3
CLASS B. GROUP I. Na ₂ HPO ₄ in water.	1.84	4.7	5.3	5.5	4.8	4.1	5.2	5.4	5.7	5.0	5.6	6.3	3.9
GROUP II. No P ₂ O ₅ supplement	2.1	2.7	2.3	2.2	1.9	2.3	2.2	3.5	2.7	2.8	4.3	4.8	2.5

Discussion.

It will be noticed from the table and the figures that the inorganic phosphorus in the blood of all the groups given a P₂O₅ supplement, rose soon after the beginning of the experiment. The inorganic phosphorus of the control animals remained low. Periodic rises are to be observed which can probably be associated with the P₂O₅ content of the grazing. These variations are also reflected in the analyses of blood of the groups receiving phosphorus supplements.

It will be of interest to give the November, 1929, determinations of the individual animals in Class B, i.e. before the experiment was commenced:—

Group I, Animal No. 2308	2.9	mg.	Inorg. P	per 100 c.c.
2416	1.75		"	"
2426	1.45		"	"
2442	1.54		"	"
2454	1.54		"	"
Average	1.83		"	"
Group II, Animal No. 2323	3.1		"	"
2356	1.8		"	"
2384	2.0		"	"
2397	1.9		"	"
2427	1.6		"	"
Average	2.1		"	"

Animals Nos. 2426, 2442 and 2454 showing the lowest amount of inorganic phosphorus in the blood were actually showing the most pronounced symptoms of Styfsiekte.

From these figures it may be said in a general way that values for inorganic phosphorus lower than 3.5 mg. per 100 c.c. blood indicate a condition of aphosphorosis.

It was impossible to correlate these figures with osteophagia. The inorganic phosphorus content of the blood of the control animals was lower throughout the whole period, and it would have been an easy matter at any time to distinguish the control groups from the others. In other words, for the purpose of diagnosing aphosphorosis this method, i.e. the determination of the inorganic phosphorus content of the blood, would have been satisfactory at any stage in the experiment. Unfortunately as much cannot be said for the pica testing method. It does not provide a way of ascertaining whether animals are on a phosphorus-deficient diet, but only aims at a way of finding out whether animals will pick up carcass debris, possibly infected, if given access to it and in that way contract Lamsiekte. It becomes abundantly clear that in a study of the great subject of aphosphorosis in the Union, pica tests have very limited value but that blood analysis gives one an insight into the phosphorus metabolism of the animal and provides a way of actually diagnosing aphosphorosis. Du Toit, Malan and Rossouw (1930) pointed out in their experiment that even the degree of phosphorus deficiency may be ascertained from blood analysis. Sheep on a diet extremely deficient in phosphorus showed lower figures for blood phosphorus than those on a phosphorus-sufficient diet.

A SUGGESTION FOR THE ARRANGEMENT OF RESERVOIRS, ETC., FOR ADMINISTERING PHOSPHORUS TO ANIMALS IN THE DRINKING WATER.

On Fig. 8, a sketch is given showing how the reservoirs, trough, etc., could be arranged for carrying out this method of feeding phosphorus to animals under ordinary farming conditions. In very many cases farmers have erected corrugated iron or cement reservoirs, and animals are watered from these. The reservoirs vary in capacity, largely depending upon the amount of water available, and usually hold about 15,000 gallons. It is suggested that an additional reservoir be erected. The piping from the windmill can be arranged as shown on the sketch, so that either reservoir can be filled. When one reservoir is full, the water soluble phosphate is added in the amount decided upon. The water from this reservoir is now supplied to the animals by the arrangement of the stopcocks as indicated. If camps are conveniently situated, a series of water troughs in the different camps can be filled from this central water supply. While the water in one reservoir is being used, the other is being filled, and can be used when the water becomes exhausted in the other. It is further suggested that such reservoirs should be provided with a scale graduated at convenient intervals, so that the volume of water in a reservoir can at any time be determined. It will often happen that

on account of low wind the treated water in one reservoir will have been used up while the other one will not yet be full. This half-filled reservoir can be used and the correct amount of phosphate added if the volume of water contained is known.

This suggested method of arranging the water supply is made especially in view of the conditions which exist in Bechuanaland. The topography of that area is particularly flat. If a special phosphate-mixing tank for the water is to be installed, it will in many cases involve much additional expense, as the supply reservoirs would have to be erected on a higher level than the mixing tank, so that sufficient pressure is obtained to fill it. If a reservoir is conveniently situated, no objections could be raised against the erection of a mixing tank of convenient capacity for adding the phosphates to the water prior to its being taken by the animals.

The addition of the small amount of acid to the water to obtain complete solution of the phosphates, as for example when di-sodium phosphate is used in water like that at Armoedsvlakte, containing a large amount of calcium, it will have a negligible effect on the cement or corrugated iron of which the tank is made. The acid is immediately neutralized by the calcium salts present and very little is available to corrode the materials of the reservoir, piping and troughs. From the determinations which have been made on the P.H. values of the water, it will be noticed that the reaction of the solution after the addition of the acid is practically neutral. Farmers usually paint these tanks with bituminous paint. A thin coat of this material will be quite sufficient to prevent the corrosive action of any acid that may still be present. The same remarks will apply in the event of any of the acid phosphates being used, e.g. mono-sodium or mono-ammonium phosphate.

To erect a circular corrugated iron tank with a capacity of 15,000 gallons should cost approximately £20. The expense incurred by farmers adopting this method of administering phosphates to their animals constitutes an insignificant outlay of capital.

SOME FURTHER ECONOMICAL AND PRACTICAL CONSIDERATIONS OF THE METHOD OF ADMINISTERING PHOSPHATES TO ANIMALS IN THE WATER.

COMPARISON OF THE COST OF WATER SOLUBLE PHOSPHATES AND BONEMEAL.

As already mentioned under the heading "Selection of material, etc., the use of the mono-basic water soluble phosphates is to be preferred, as in this case no additional acid need be added to the water. It is doubtful whether water with such a high alkaline value as to interfere with the solution of the mono-sodium or mono-ammonium phosphate would be suitable for stock. The fact that these salts will go into complete solution excludes the additional cost and danger of using the acid. These mono-basic salts contain a high percentage of P_2O_5 . A high grade mono-ammonium phosphate is being prepared by a firm of chemical manufacturers and contains 56.5 per cent. water soluble P_2O_5 . Such a highly concentrated P_2O_5 salt reduces cost of transport.

This high-grade mono-ammonium phosphate is at the moment quoted by the firm in question at £18. 10s. per English ton (2,240 lb.) landed on boats in English ports, and should not cost more than £25 in any district of the Union. This price will now be considered in making a comparison with the cost of bonemeal.

Farmers have to pay about £8 per ton for bonemeal. If 3 oz. bonemeal is fed daily to cattle on weekdays, it costs the farmer 5s. per animal per annum for the bonemeal used. As bonemeal contains approximately 22 per cent. P_2O_5 , the 3 oz. of bonemeal contains 18.81 gm. P_2O_5 .

For 5s. the farmer can buy:

$\frac{2240 \text{ by } 5}{25 \text{ by } 20}$ i.e. 22.4 lb. mono-ammonium phosphate (at £25

per English ton), and if he feeds this amount to an animal daily on 365 days of the year, his animals will get 3584/3650 of an ounce or very nearly 1 oz. daily. This ounce of ammonium phosphate (56.5 of P_2O_5) contains 16.1 gm. P_2O_5 .

In the case of bonemeal, therefore, 5s. provides 18.81 gm. indifferently available P_2O_5 to the animal on 6 days of the week, or 16.12 gm. per diem, practically the identical amount of P_2O_5 supplied by 1 oz. mono-ammonium phosphate, in a very available form.

In this experiment, where the phosphorus was administered in the drinking water the results described were obtained by giving 1.56 oz. di-sodium phosphate, of a 20 per cent. P_2O_5 content, per diem. These animals received 10.08 gm. P_2O_5 daily. An additional 6 gm. can be administered by using the mono-ammonium (of which the price is known) before the cost of the material exceeds the cost of the 3 oz. bonemeal.

As this method entails a minimum amount of labour, farmers who adopt it could reduce their labour expenditure considerably.

The disadvantages of the present methods of administering P_2O_5 , already enlarged upon, will be removed, and incidentally the farmer will bestow more care upon the water supply of his stock. In the various campaigns which veterinarians and others have launched against verminosis in stock, they emphasized the necessity of a more hygienic water supply.

Bonemeal is now extensively fed to sheep in South Africa, for there is evidence that a deficiency of phosphorus has adverse effects on this animal also. (Bekker and Rossouw, 1930), Du Toit, Malan and Rossouw (1930). The method of administering phosphates through water can be very successfully adopted for sheep and even where they are kept on the same farm as cattle, the amount of phosphate in the water specially made up for the latter will in all probability suit the requirements of the sheep too. No definite information is available at present, however, on the comparative amounts of water consumed by cattle and sheep grazed under similar conditions.

It must be pointed out that, while this method of administering P_2O_5 to stock in the drinking water can readily be adopted where the water supply is under easy control, it will probably be impossible to make use of it in districts with a high rainfall and much open water. Such areas, however, are usually highly cultivated and farms there are small, so that any of the other methods in use for giving mineral supplements to stock, e.g. feeding these supplements with the food in the byre, will be practicable.

SUMMARY AND CONCLUSIONS.

1. The disadvantages of the present methods of administering phosphorus supplements, especially in the case of veld-fed cattle, are discussed. With the "hand-feeding" method it is considered that the frequent handling interferes with the natural grazing habits of the animals, increases katabolism, leads to deterioration of the veld and involves a considerable amount of labour. With regard to "licks" the main disadvantages are the uncertainty of animals getting their phosphorus supplement, and the higher costs of feeding bonemeal in this way.

2. A new method of administering phosphorus-containing supplements to animals through the drinking water is suggested. This method could be easily adopted in many parts of South Africa, especially in the arid and semi-arid regions where there is a phosphorus deficiency, as the water used for stock is derived mainly from boreholes.

3. Di-sodium phosphate, with 20 per cent. P_2O_5 content was selected merely on account of its being available in sufficient amounts. This salt was dissolved in the water in such a concentration that the animals received approximately 1.5 oz. (i.e. 10 gm. P_2O_5) per head per diem.

The concentration was determined according to the average amount of water consumed by the experimental animals over the experimental period (11.11.29 to 31.12.30).

In order to obtain complete solution of the phosphates a small quantity of sulphuric acid was added to the water, viz., 1 c.c. of 90 per cent. commercial H_2SO_4 to each gallon of water. A trace of copper sulphate (1 in 250,000) was added to the water containing the dissolved phosphates to inhibit the growth of algae.

Small laboratory tests indicate that if mono-sodium or mono-ammonium phosphate is used the addition of the sulphuric acid is not necessary even in the Armoedsvlakte water which contains a large amount (120 mg. CaO and 80 mg. MgO per litre) calcium and magnesium salts in solution.

4. Experimental work with *two* classes of cattle is described:

Class A—nine-month old weaned calves.

Class B—three-year old oxen, which had been previously used in an experiment and had not been given a phosphorus supplement. The animals of *Class A* were divided into various groups of eight in

each. Cattle of approximately the same age, type and sex were included in the different groups. The control group of this class weighed appreciably more than the other groups. This selection was purposely made so that no doubt could arise about the inferiority of this group. The average weight of all the other groups was very nearly the same. The control group (No. IV) received no phosphorus supplement.

The other three groups received the following supplements:

Group I.— $1\frac{1}{2}$ oz. di-sodium phosphate (20 per cent. P_2O_5) daily through the drinking water.

Group II.—2 oz. bonemeal (after 6 months, doses increased to 3 oz.) (22 per cent. P_2O_5) dosed daily except on Sundays.

Group III.— $1\frac{1}{2}$ oz. di-sodium phosphate (20 per cent. P_2O_5) given as in Group II.

Class B.—Divided into 2 Groups:—

Group I.—7 animals given $1\frac{1}{2}$ oz. Na_2HPO_4 with Group I of Class A.

Group II.—6 animals no supplement given (controls).

5. From the results obtained from the treatment of these animals the following conclusions are made:—

- I. The water containing the Na_2HPO_4 , H_2SO_4 and trace of $CuSO_4$ was taken by the animals without any trouble whatsoever, and they preferred the treated water to the other water on the farm.
- II. The dosage of the Na_2HPO_4 could be controlled to a remarkable degree of accuracy by giving it in the drinking water. It was intended to give 1.5 oz. daily; actually an average amount of 1.56 oz. was consumed by the animals during the progress of the experiment.
- III. In Class A the animals receiving their phosphorus through the water showed the greatest improvement in weight, in spite of the fact that Anaplasmosis considerably interfered with the weight of some of the animals in this group (Group I). This improvement over and above that in the other groups, is considered to be largely due to the fact that these animals were handled to a minimum extent. In Class B the animals in Group I improved remarkably in condition when compared with the controls (Group II).
- IV. In both classes no animals in the groups receiving a phosphorus supplement ever developed Lambsiekte. Cases of Lambsiekte occurred in both control groups. Although separately grazed, it is considered that the groups which received the phosphorus supplement in the drinking water were exposed at least to the same degree of risk of infection.

- V. Osteophagia as indicated by "pica tests" was reduced to a satisfactory basis in Group I and II of Class A and Group I of Class B. In Group III of Class A (Na_2HPO_4 dosed), pica was not effectively reduced. This is probably on account of the smaller dose administered; actually this group were given 1.29 oz. Na_2HPO_4 compared with 1.56 in Group I (Na_2HPO_4 in water).
- VI. Styfsiekte, present in some of the animals of Group I of Class B, disappeared some time after the phosphorus was given in the water.
- VII. Inorganic phosphorus determinations in the blood of the various groups indicate the persistently greater amount of inorganic phosphorus present in the blood of the animals getting the phosphorus supplements than in that of the controls. The value of these determinations to diagnose a phosphorus deficiency is again demonstrated.

6. A suggested practical method of adopting the system of administering phosphorus and even other minerals to animals is given. It is pointed out that information should be accumulated in order to arrive at a suggested "Standard concentration of phosphorus in the drinking water", which should suit the phosphorus requirements of different classes of cattle and sheep on a farm in a phosphorus deficient area. For *dry mature cattle* 6 gallons is the quantity of water tentatively suggested as a possibly convenient amount to base this "Standard concentration" on, and it is suggested that to this quantity of water 10 gm. P_2O_5 should be added. For *weaned calves, young growing stock and cows in milk* a more concentrated solution seems to be required, and it is suggested that for these animals 10 gm. should be added to 4 gallons water.

7. The economical and practical advantages of the system of administering P to animals in the drinking water is discussed, and compared especially with the methods of feeding bonemeal as a source of phosphorus. The most important advantages are:—

- (a) Less labour is required.
- (b) It is not necessary to continually collect and dose the cattle, which means that the natural grazing habits are not disturbed and less trampling of the veld takes place; this brings about a greater carrying capacity of the veld. Unnecessary expenditure of energy by the animals is avoided.
- (c) Animals will tend to regulate the amount of P they require themselves as they consume different quantities of water according to their size and condition.
- (d) The animals can utilize more P in the water soluble phosphates than in the case of bonemeal.
- (e) For the same outlay more available P can be given to the animals by using those water soluble phosphates of which the price is known, than in the case of bonemeal.
- (f) The adoption of this system of giving P to stock will be conducive to a better and more hygienic control of the drinking water of stock.

8. It is considered that the method of administering phosphorus to animals in the water is probably not practical in districts where there is a large amount of open water, i.e. not easily controlled.

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REFERENCES.

- BEKKER, J. G., and ROSSOUW, S. D. (1930). A Note on some Conditions in Sheep in the Strandveld of the Bredasdorp District. *16th Rept. Dir. Vety. Serv., U. of S.A.*, pp. 293-300.
- CRICHTON, J. A. (1930). The Mineral Requirements of Dairy Cattle. *Jnl. of Dairy Research*, Vol. 2, No. 1.
- DU TOIT, P. P., and BISSCHOP, J. H. R. (1929). The Breeding of Cattle on Phosphorus Deficient Veld. *15th Rept. Dir. Vet. Serv., U. of S.A.*, pp. 1059-1166.
- DU TOIT, P. J., and GREEN, H. H. (1930). Di-calcium phosphate in Nutrition. *16th Rept. Dir. Vety. Serv., U. of S.A.*, pp. 267-286.
- DU TOIT, P. J., MALAN, A. I., and ROSSOUW, S. D. (1930). Phosphorus in the Sheep Industry. *16th Rept. Dir. Vety. Serv., U. of S.A.*, pp. 313-326.
- ECKLES, C. H., BECKER, R. B., and PALMER, L. S. (1926). A Mineral Deficiency in the Rations of Cattle. *Minnesota St. Bull.*, 229, 4.
- GREEN, H. H. (1927). Osteophagia and Phosphorus Deficiency in Relation to Lambs. *11th and 12th Rept. Dir. of Vety. Educ. and Res.*, Part 2, pp. 1211-1248.
- GREEN, H. H. (1928). Studies in Mineral Metabolism IV. *Jnl. of Agric. Sc.*, Vol. 18, Part 3, pp. 372-375.
- HENRICI, M. (1928). The Phosphorus Content of the Grasses in Bechuanaland in the course of their development. *13th and 14th Rept. of Dir. of Vet. Educ. and Research*, pp. 1077-1208.
- HENRY, W. A., and MORRISON, F. B. (1922). Feeds and Feeding. 18th ed.
- HUTCHEON, D. (1884). Report of the Colonial Veterinary Surgeon. 1-15.
- MALAN, A. I. (1930). Mineral Metabolism and Blood Analysis. *16th Rept. Dir. Vety. Serv., U. of S.A.*, pp. 307-311.
- MALAN, A. I. (1930). Mineral Metabolism and Blood Analysis. *16th Rept. Vety. Serv.*, pp. 1077-1208.

- MEIGS, E. B., and WOODWARD, T. E. (1921). Influence of Calcium and Phosphorus in Feed. *Jnl. of Dairy Sc.*, Vol. 4, p. 185-217.
- ROSSOUW, S. D. (1930). Effects of Bonemeal Feeding on the Phosphorus Partition of the Blood of Sheep. *16th Rept. Div. Vety. Serv.*, U. of S.A., pp. 301-306.
- STAPLES, and TAYLOR, A. J. (1929). Studies in Pasture Management. *S.A. Jnl. of Sc.*, Vol. 26, pp. 139-153.
- THEILER, A. (1920). The Cause and Prevention of Lamsiekte. *Jnl. Dept. of Agric.*, U. of S.A., Vol. 7, No. 3, pp. 221-244.
- THEILER, A., GREEN, H. H. and DU TOIT, P. J. (1924). Phosphorus in the Livestock Industry. *Jnl. Dept. of Agric.*, U. of S.A., Vol. 8, No. 5.
- THEILER, A., GREEN, H. H., and DU TOIT, P. J. (1927). Minimum Mineral Requirements of Cattle. *Jnl. Agric. Sc.*, Vol. 17, Part 3, pp. 291-314.
- WEDEMANN, W. (1931). Kann phosphorsauer Futterkalk, der bei der Verarbeitung milzbrandsporenhaltigen Knochenmaterials in Gelatinefabriken gewonnen wird, noch lebensfähige Milzbranderreger enthalten? *Arch. wiss. u. prakt. tierhkl.* 63. Band. 3. Heft., pp. 215-229.
- "Final Report of the Drought Investigation Commission," October, 1923, U. of S.A.
- Official Year Book of the Union of South Africa. No. 11, 1928-1929.

APPENDIX.



Plate 1.—Photo of tank and trough used.

Fig. 2.—Weight curves (lb.), Class A.

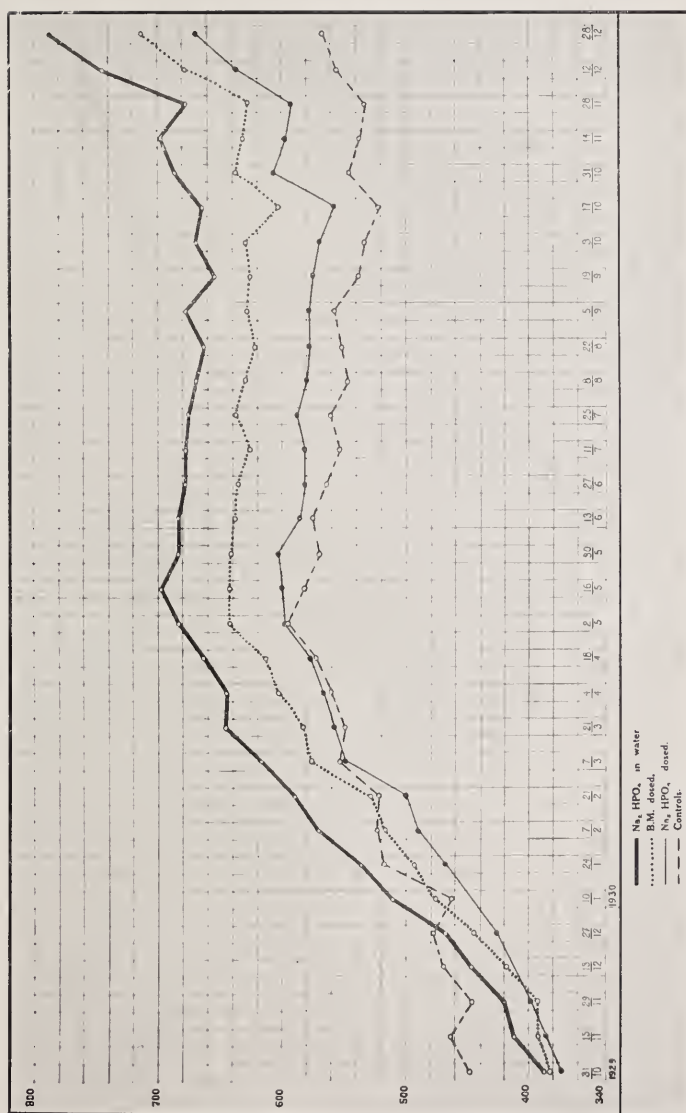
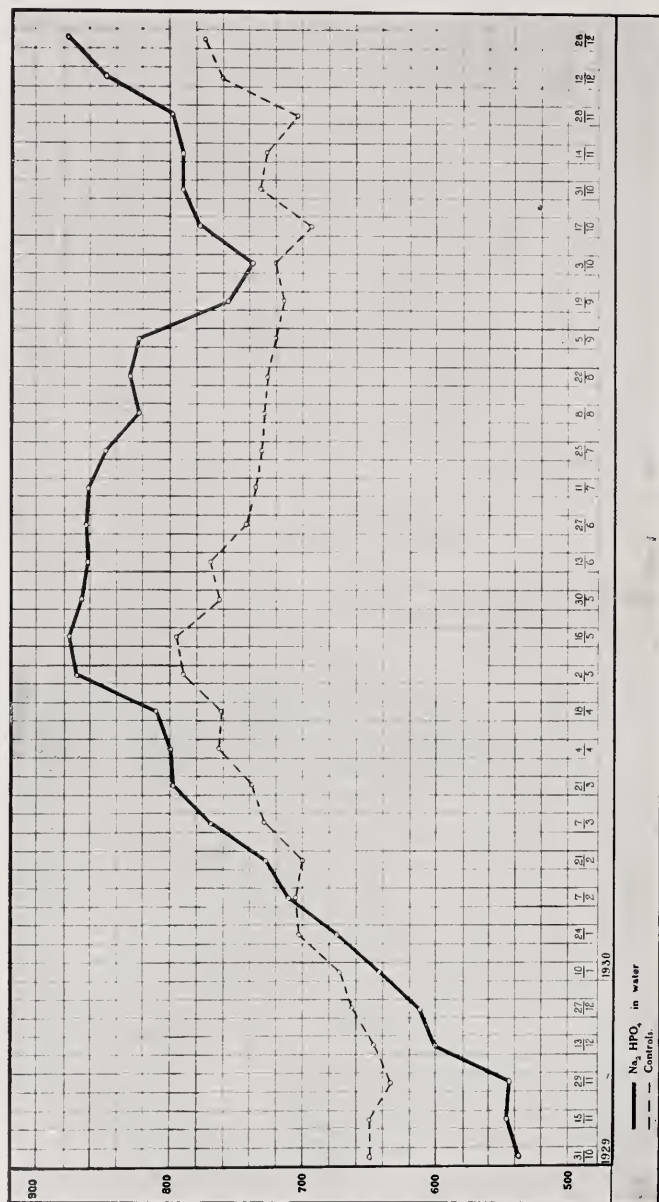
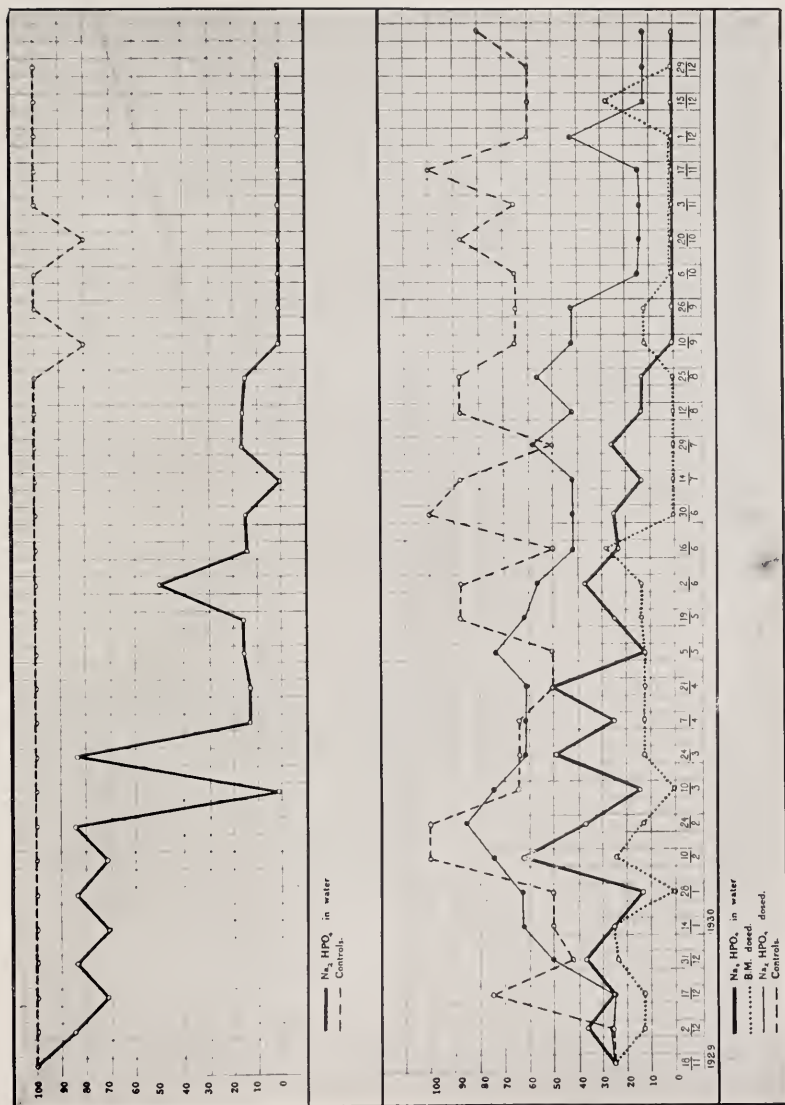


Fig. 3.—Weight curves (lb.), Class B.



Figs. 4 and 5.—Percentage rotten bone cravers in Class B (top) and A.



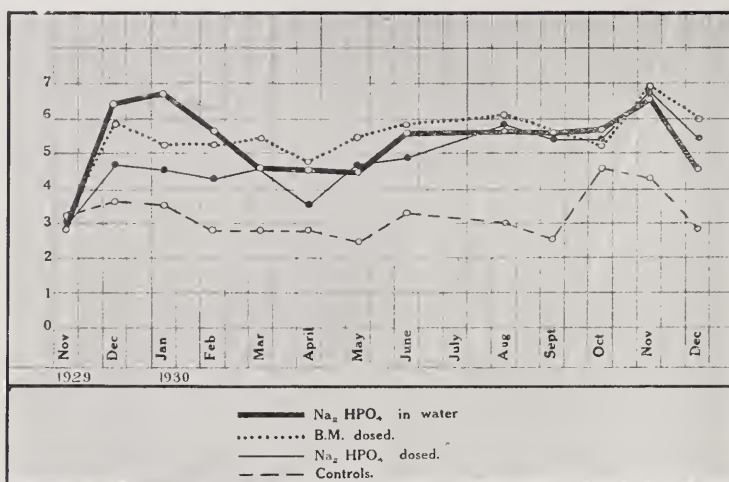


Fig. 6.—Inorganic phosphorus (mg.) per 100 c.c. blood, Class A.

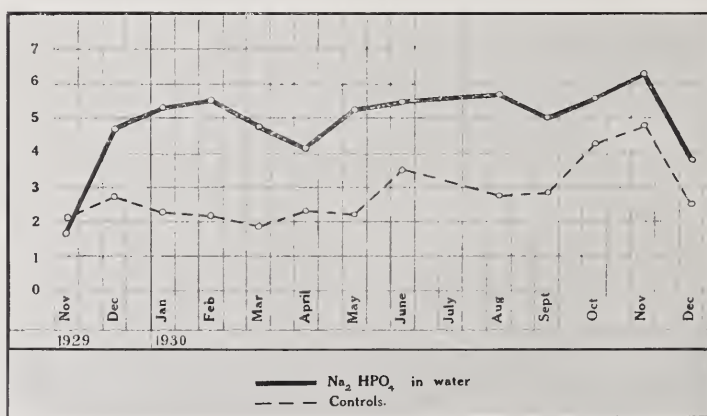
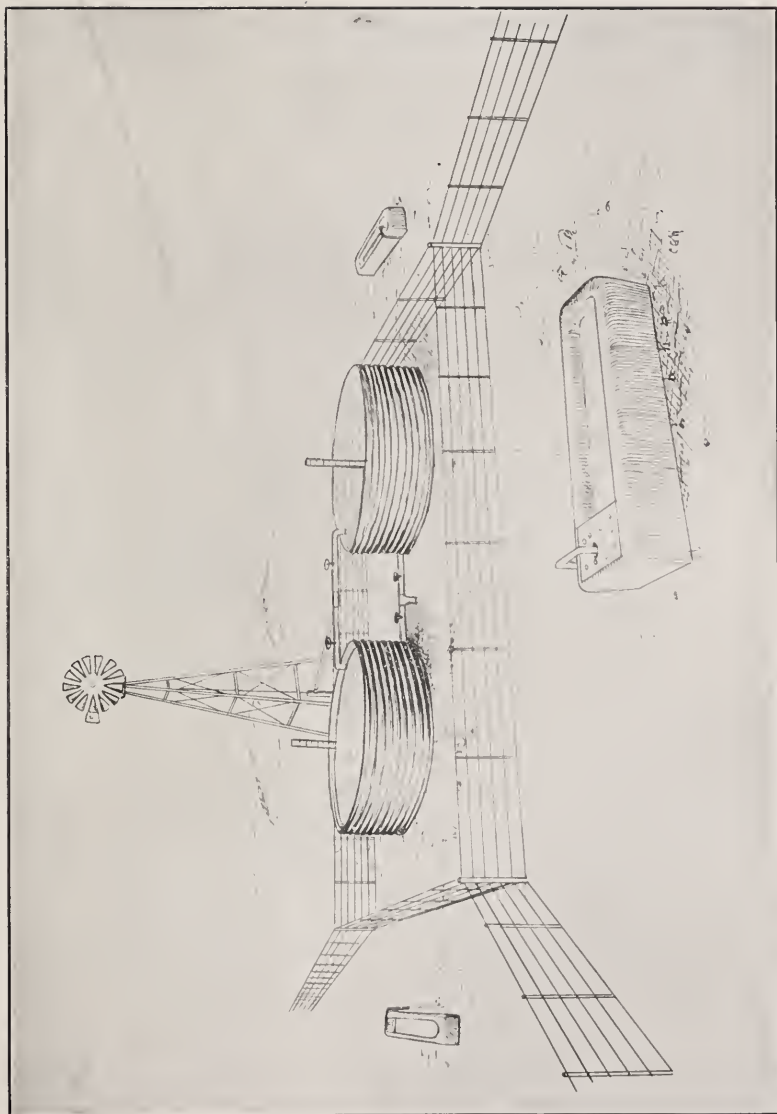


Fig. 7.—Inorganic phosphorus (mg.) per 100 c.c. blood, Class B.

Fig. 8.—Schematic illustration of suggested arrangement of water supply.



CAUSE, DATES OF DEATH, ETC., OF ANIMALS IN VARIOUS GROUPS.

It has been said that the occurrence of Anaplasmosis in the groups receiving the phosphorus in the drinking water considerably interfered with their weights, and that Lamsiekte occurred only in the control groups. The following records of these conditions are appended:

Class A.

Group I (Na_2HPO_4 in water):

No. 3043. Died 16.9.30 Anaplasmosis.

No. 3082. Anaplasmosis diagnosed on 22.8.30 and animal died on 30.9.30.

No. 3053. Anaplasmosis diagnosed on 27.4.30 but recovered.

Group II (Bonemeal dosed); and

Group III (Na_2HPO_4 dosed): No deaths.

Group IV (Controls) No. 3017, died 21.12.29 Lamsiekte.

No. 3062, died 13.1.30 Lamsiekte.

No. 3010, died 18.12.30 Lamsiekte.

Class B.

Group I (Na_2HPO_4 in water):

No. 2426, died 25.4.30 Anaplasmosis.

No. 2442, died 23.9.30 Anaplasmosis.

No. 2454, Anaplasmosis diagnosed on 13.8.30, but recovered.

No. 2405, Anaplasmosis diagnosed on 24.4.30, but recovered.

Group II (controls):—

No. 2384, died 29.11.29 Lamsiekte.

In Class A, three animals, Nos. 3074, 3060 and 3064 were taken out of Groups I, II, and III, on 28.5.30, for the formation of another group in the Comparative Phosphate Test.

RAINFALL RECORDS AT ARMOEDSVLAKE.

	1929.	1930.
January	1·58" on 7 days.	2·67" on 12 days.
February	1·52" on 5 days.	2·08" on 6 days.
March	3·46" on 9 days.	2·64" on 7 days.
April	1·22" on 1 day.	0·99" on 3 days.
May	0·18" on 1 day.	0·05" on 1 day.
June	0·00" on 0 days.	0·07" on 1 day.
July	0·24" on 1 day.	0·00" on 0 days.
August	0·65" on 3 days.	0·05" on 1 day.
September	1·08" on 8 days.	0·00" on 0 day.
October	0·00" on 0 days.	0·97" on 5 days.
November	2·56" on 8 days.	1·38" on 5 days.
December	3·36" on 11 days.	1·75" on 8 days.
Total	15·85"	12·65"

The average rainfall of Armoedsvlakte over last 7 years, 15·5".

Studies in Mineral Metabolism XXV.

The Effect of Calcium and Magnesium Supplements on the Growth of Merino Sheep.

By J. W. GROENEWALD, M.Sc.(Agric.), Research Officer,
Onderstepoort.

CALCIUM as a supplement in live stock rations has not received much attention in South Africa due mainly to the abundance of this substance in the majority of our soils. Its great importance in the livestock industry, however, fully justifies more extensive studies than those of Du Toit, Malan and Groenewald (1931), where five ewes showed no serious set-back after two years on a ration considered to be deficient in calcium.

There is likewise no fear of a magnesium deficiency in South Africa, as it is even more widely distributed in nature than is calcium, and is not required in such large quantities. Whether magnesium supplements exercise good or bad influences on animals is at present a question of uncertainty. Becka (1929) has shown that calcium retention was favourably influenced by increased intake of magnesium. Haag and Palmer (1928) found that in the rat a high intake of magnesium had a depressing effect on calcium retention. Hart, Steenbock and Bohstedt (1927) working on pigs, came to the conclusion that increased magnesium had no adverse effects unless given in quantities that would cause digestive disturbances.

The present trial was, therefore, started with three groups of healthy 2-tooth Merino ewes and three groups of 4-tooth wethers, each group consisted of five sheep. All the sheep were shorn at the commencement of the trial, and put into an ordinary earth floor paddock where rain water was given *ad lib.*, and the following ration fed daily per sheep in a common trough:—

0·5 lb. of veld hay.

1·0 lb of crushed maize.

8 ounces of common salt.

The calcium, magnesium and phosphorus content of the ration, as well as the amount of these substances supplemented are as follows:—

Group I *Controls.*

Calcium in ration..... 0.09 gm.

Group II *Calcium supplement.*

Calcium in ration..... 0.09 gm.

Calcium supplement (7 gm. CaCO_3)..... 3.9 gm.

TOTAL.....=3.99 gm. Ca.

Group III *Magnesium supplement.*

Magnesium in ration..... 0.63 gm.

Magnesium supplement [3.5 gm. $\text{Mg}(\text{OH})_2$]=1.47 Mg.

TOTAL.....=2.1 gm. Mg.

The phosphorus intake in their feed amounted to 2.55 gm. daily per sheep. The amount of minerals supplemented was based on the intake of sheep on good English hay, assuming that $2\frac{1}{2}$ lb. is consumed daily per sheep. In addition to the magnesium supplement, Group III received the same amount of calcium as did Group II. All the mineral supplements were dosed daily except Sundays. The monthly weights of the wethers are shown in Figure 1.

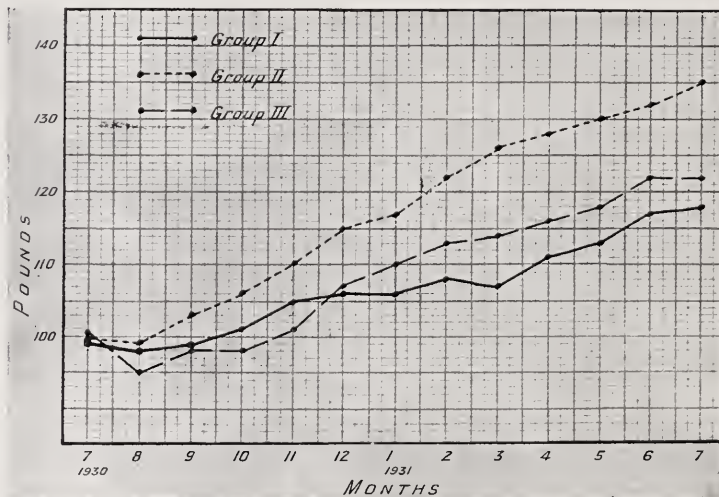


Fig. 1. Group weights of wethers.

The very pronounced increase in the monthly average weights of Group II, is very noticeable. This group shows an average gain of 17 lb. over and above the control group, and a gain of 13 lb. on Group III (Magnesium supplement).

In order to study the manner in which the ewes reacted to these supplements Fig. 2 is given:—

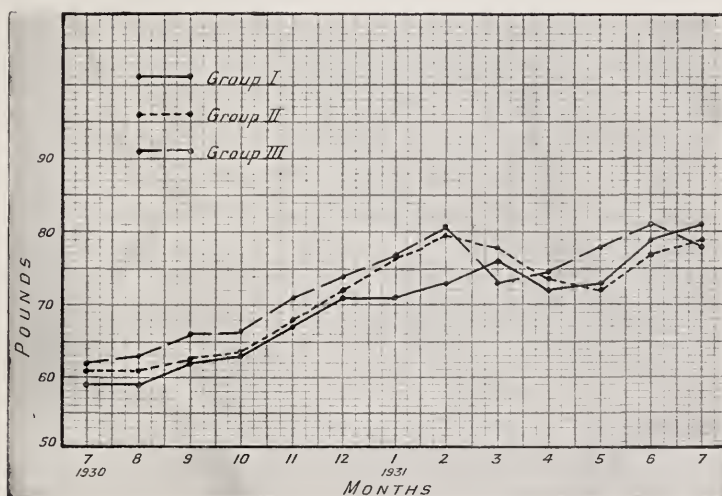


Fig. 2. Group weights of ewes.

As may be seen from the weight curves in Fig. 2, there was no significant difference between the average monthly weights of the various groups throughout the experimental period. A fact which at once calls for the interpretation of the lambing chart in Table 1.

TABLE 1.
LAMBING CHART.

Group.	No.	Date of Lambing.	Remarks.
I.....	26145	—	—
	26730	30.3.31	Lamb normal.
	26148	—	—
	28689	30.3.31	Died (31.3.31).
	26160	—	—
II.....	26162	10.3.31	Lamb normal.
	26147	16.3.31	Born dead.
	26151	4.4.31	Lamb normal.
	28801	—	—
	26144	14.4.31	Lamb normal.
III.....	26146	—	—
	26150	12.3.31	Lamb normal.
	28597	27.2.31	Died (15.3.31).
	26155	18.4.31	Lamb normal.
	26161	27.5.31	Aborted.

10.5.31 all lambs taken away from ewes.

The fleeces of the wethers receiving calcium each averaged 9 lb. more than those of the controls, while the fleeces of the sheep receiving magnesium actually yielded a lb. less on the average than the controls. The fleeces of the ewes receiving calcium, on the other hand, averaged 1.7 lb. less than those of the controls and 0.6 lb. less than those of the group receiving magnesium supplement. Details of the wool weights are given in Table 2.

TABLE 2.
WOOL WEIGHTS IN POUNDS.

WETHERS.				EWES.			
Group.	No.	Fleece Weight.	Average Group Weight.	Group.	No.	Fleece Weight.	Average Group Weight.
I....	26825	10.3	} 10.06	I....	26145	8.6	} 8.50
	26834	9.0			26730	9.8	
	26833	11.6			26148	9.0	
	26816	10.2			28689	7.2	
	26812	9.2			26160	7.9	
II....	26821	11.4	} 10.92	II....	26162	6.5	} 6.88
	26809	11.7			26147	9.5	
	26820	9.3			26151	5.4	
	26811	12.9			28801	7.1	
	26803	9.3			26144	5.9	
III....	26824	9.2	} 9.00	III....	26146	8.0	} 7.42
	26830	7.3			26150	6.7	
	26813	Died*			28597	8.7	
	26818	9.5			26155	6.5	
	26823	10.0			26161	7.2	

* Death due to urethral calculus.

CONCLUSIONS.

There are strong indications as is seen from Fig. 1 that the group of wethers receiving a calcium supplement have gained steadily on the other groups, notwithstanding the fact that these sheep weighed about a hundred pounds and were full-grown when the trial was started. The group receiving the magnesium supplement made very poor gains, averaging only four pounds better than the control group.

The unscoured wool weights are also slightly in favour of the calcium supplement, while the total yield of wool was least in the group receiving the magnesium supplement.

According to Fig. 2, there is no apparent difference in the three groups of ewes in regard to group gains in weight. It is practically impossible, however, to measure the influence exercised on the various groups by gestation and lactation. The above factors undoubtedly have greatly contributed to the unbalanced condition ultimately derived in this trial. The greater strain must have been borne by the

group receiving the calcium. In this group four lambs were born, three of these were healthy and remained with the ewes, while only one lamb was suckled in the control group and two lambs in the magnesium group.

It is suggested that future work of this nature be carried out on concrete floors in order to avoid earth eating by the sheep. The element of reproduction is also best left out of work of this nature as it considerably endangers the chances of uniformity within the groups.

SUMMARY.

1. It was found that the supplementation of 7 gm. CaCO_3 daily had an apparently beneficial effect on growth in Merino sheep.

2. A group of sheep receiving magnesium supplement did not make as rapid gains as did the calcium supplement group.

3. No conclusions could be drawn from the ewes because of the lamb crop failure.

ACKNOWLEDGMENTS.

The writer appreciates the facilities placed at his disposal by Dr. P. J. du Toit, and wishes to express his indebtedness to Dr. A. I. Malan for his valuable advice and encouragement.

REFERENCES.

- BECKA, J. (1929). Magnesium als Regulator des Calciummetabolismus. *Prager Archiv. für Tiermedizin*, Teil A, Heft 1, 2, 3, pp. 253-259.
- DU TOIT, P. J., MALAN, A. I., and GROENEWALD, J. W. (1931). Studies in Mineral Metabolism, XIII, 17th Rept. Dir. Vet. Serv. and Anim. Indust., pp. 453-472.
- HAAG, J. R., and PALMER, L. G. (1928). The Effect of Variations in the Proportions of Calcium, Magnesium and Phosphorus contained in the Diet. *Jnl. Biol. Chem.*, Vol. 76, p. 367.
- HART, E. B., STEENBOCK, H., and BOHSTEDT, G. (1927). What Minerals for Wisconsin Livestock? *Arg. Exp. Sta. Bull.*, 390, pp. 12.

Studies in Mineral Metabolism XXVI. The Effect of Fluorine on Pregnant Heifers.

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INTRODUCTION.

Rock phosphate was included in a group of feeding supplements for stock at Armoedsvlakte in order to study its effect on the health and growth of animals and to arrive at an idea of the cause of its detrimental effect, if any became apparent in the manner observed by a number of workers—Forbes (1921), Reed and Huffman (1928) and (1930), McCollum (1925), Taylor (1929), and Tolle and Maynard (1929).

The results of the Armoedsvlakte experiment are presented in detail by Malan and du Toit (1932) in another article in this Report, and are merely mentioned here on account of the toxic symptoms which the animals developed during the course of the investigation.

These symptoms were identical with those produced in an experiment on the toxicity of sodium fluoride to be reported on in this paper and were regarded to be due to fluorine poisoning.

Simultaneously with the rock phosphate experiment two Friesland grade heifers (Nos. 3674 and 3676) were placed on a diet of which the fluorine content was made approximately equal to that of the daily dose of rock phosphate by the addition of sodium fluoride. Observations could thus be made upon the effect of feeding rock phosphate to a group of cattle under ranching conditions on the one hand and upon that of feeding fluorine as sodium fluoride to two stall-fed heifers on the other. The results of the latter investigation are presented in this article.

LITERATURE.

From the available literature it appears that much work has been done on the effects of administering fluoride orally. Schwyzer (1903) found that rats decreased in weight and developed poor appetites after the addition of sodium fluoride to their diet. In 1914 the same author concluded that "Fluorpräparate, in kleiner Dose chronisch zugeführt sind giftig, selbst bis unter 1 mg. pro Tag pro Kg. Körpergewicht, und sollten sowohl für die Ernährung von Menschen als die Fütterung von Schlacht- und milchvieh gänzlich ausgeschaltet werden."

Bethke (1930) reported that swine given sodium fluoride in the ration lost weight. The fluoride content of the bones increased. These results with swine were confirmed by MacLure (1931). Hupka and Götze (1931) reported cases of fluorine poisoning in cattle. The animals developed lameness, sore feet, large painless swellings on the costal arch and sometimes round the pastern joints. Feeding cattle with sodium fluoride and sodium fluosilicate, 3-6 grams, caused acute poisoning with painful disturbance in health. The animals recovered 8-10 days after the last dose had been administered.

EXPERIMENTAL WORK.

Four grade Friesland heifers two years old were placed on the following basal ration:—

- 4·5 lb. veld hay.
- 4 lb. crushed maize.
- 5 lb. rolled maize endosperm or Fanko.
- 20 grams blood meal.

The amounts given represent the daily allowance per head. The small proportion of roughage was found to be suitable and did not cause digestive disturbances; the quantity could not be increased without rapidly multiplying the mineral content of the ration, and as this experiment was part of a larger experiment on the rôle of various inorganic constituents in the nutrition of heifers, the minerals in the basal ration had to be kept as low as possible. That the amount of hay is sufficient for roughage in this type of experiment is clear from a consideration of the work of Theiler, Green and du Toit (1927).

The heifers were stalled overnight and fed individually, while during the day they were allowed to exercise in a paddock with concrete floor. The composition of the ration is given below in Table I, while the following salts were added daily to the ration to ensure an adequate intake of inorganic constituents:—

- 25 grams CaCO_3 .
- 75 grams CaHPO_4 .
- 15 grams Mg(OH)_2 .
- 25 grams NaCl .
- 75 grams KCl .

The quantity of each constituent added was calculated from data giving the normal consumption of minerals for heifers on good pasture.

TABLE I.
Composition of the Basal Ration.

	Protein. gm.	Therms.	CaO. gm.	P_2O_5 . gm.	MgO. gm.	Na_2O . gm.	K_2O . gm.	Cl. gm.
Hay, 4·5-lb...	140	0·9	8·1	3·9	6·0	2·0	26·6	5·0
Maize, 5-lb...	200	4·3	0·25	10·1	2·5	2·5	8·8	1·5
Fanko 5-lb...	180	4·3	0·13	3·6	0·9	0·25	3·0	0·75
Blood meal, 20 gm.....	12·9	0·03	0·04	0·16	0·33	0·28	0·07	0·07
TOTAL....	532·9	9·53	8·52	17·76	9·73	5·03	38·5	7·3

Two of the heifers, Nos. 3676 and 3674, were dosed with a solution containing 5 grams of sodium fluoride daily, while the two others, Nos. 3639 and 3645, received the basal ration only.

Towards the end of the first month after starting, both heifers receiving the sodium fluoride showed signs of restlessness and had poor appetites, while the control heifers invariably cleaned up their daily allowance. Table 2 gives the monthly consumption of maize plus Fanko, while that of the control heifers remained constant at 300 lb. throughout the experimental period.

TABLE 2.
Maize plus Fanko consumed in lb.

D.O.B. No.	Nov., 1930.	Dec.	Jan.	Feb.	Mar.	April.	May.	June.	July.	Aug.
3676.....	293	208	199	258	229	230	201	Died 28.5.31.		
3674.....	295	189	261	283	298	274	268	249	169	223

All four heifers consumed the hay equally well.

The weight curves of the heifers are given below for the period September, 1930, to September, 1931:—

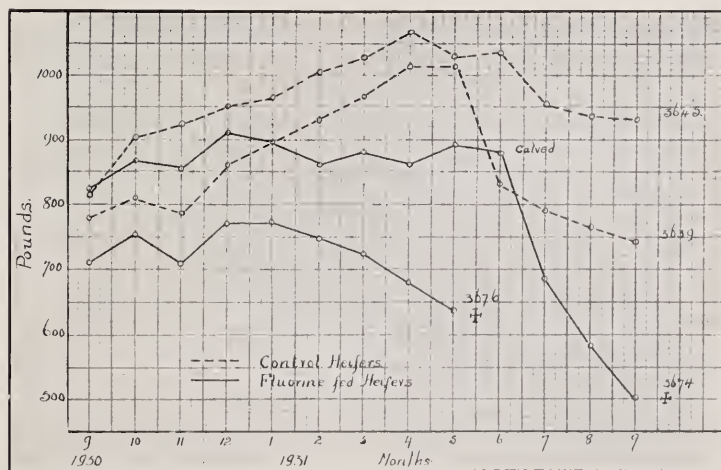


Fig. 1.

A glance at the curves indicates that the weight increases of all four animals showed little difference for the first four months of the experiment, although the table giving food consumption suggests superiority of the controls. The daily inspection of the animals left no doubt that the differences between the controls and the experi-

mental heifers such as loss of appetite and disinclination to walk on the part of the latter were bound to become more pronounced as the experiment continued. The weight curves soon began to show up the difference for, whereas the controls continued to increase in weight until lactation began, the experimental animals gradually decreased and successively developed very poor conditions with decubitus as a result of lying down most of the time. As a matter of fact No. 3674 was unable to rise without assistance towards the end of the experiment. She was destroyed in September, 1931, when her weight was only 500 lb., or 200 lb. lighter than at the beginning of the experiment. This heifer was an exceptionally fine one, and in excellent condition at the beginning of the experiment, as a glance at the photograph will show. She gave birth to a 33-lb. calf which died practically immediately after birth. The cow produced a total of 817 lb. of milk or a daily average of 8.6 lb. Lactation undoubtedly accelerated the rate of falling off in weight and was partly responsible for the ultimate weakness, although she dried up a month before death. The photographs in Figure II represent this animal at the beginning of the experiment and two months before destruction. During the last few months of the experiment this cow lay down most of the time except when feeding. She was obviously in pain when walking, although apparently standing did not cause great inconvenience and she could remain in that position for hours.

Heifer No. 3676 appeared to have fared even worse. Her loss of condition began earlier and was more rapid than that of her experimental companion. She calved, with considerable difficulty, in May, 1931; the calf weighed 42 lb. and lived only for about 30 minutes. The cow died 17 days afterwards of peritonitis, sequel of metritis. The symptoms that had developed during the last few months of the experiment were similar to those in the case of No. 3674, viz. lameness, painful and careful walking and large hard swellings on the long bones of the legs, which were always distinctly swollen around the joints. The photographs in Fig. III. represent this animal at the beginning of the experiment and just before calving. The hard knob-like swellings on the legs are distinctly visible in the second photo (right hind leg).

In contrast with the experimental animals the two control heifers grew normally, showed rapid gain in weight and retained their initial good appetite. As the weight curves in Figure I indicate that they weighed several hundred pounds heavier than the experimental heifers at the time of calving—a remarkable achievement eight months after the beginning of the experiment. The ration was too low in protein for adequate milk production, so that an anticipated sudden and rapid drop in weight was shown after calving. Nevertheless, these two controls each produced on an average 1,278 lb. of milk for the same period as No. 3674 or a daily average of 14.1 lb. The photographs in Figures IV and V show these heifers at the beginning of the experimental period and three months after calving, i.e. approximately at the time No. 3674 was killed.

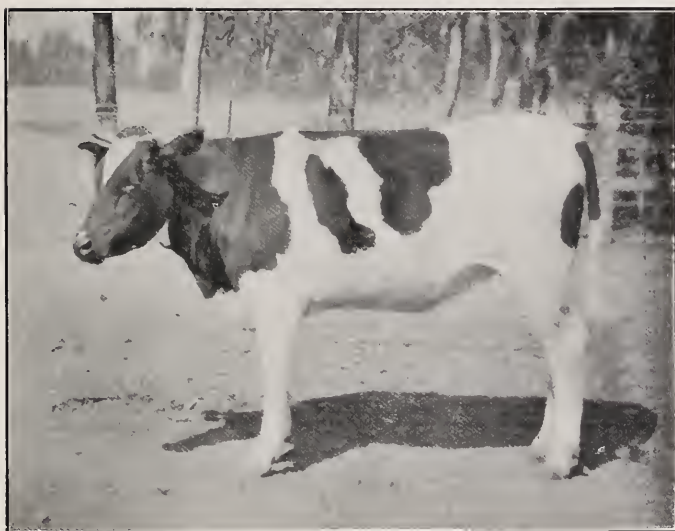


Fig. 2.—C. 3674 at beginning of experiment.



Fig. 2.—C. 3674 at end of experiment.



Fig. 3.—C. 3676 at beginning of experiment.



Fig. 3.—C. 3676 at end of experiment.



Fig. 4.—U. 3639 at beginning of experiment.



Fig. 4.—C. 3639 at end of experiment.



Fig. 5.—C. 3645 at beginning of experiment.



Fig. 5.—C. 3645 at end of experiment.



Fig. 6.—Long bones showing characteristic lesions.

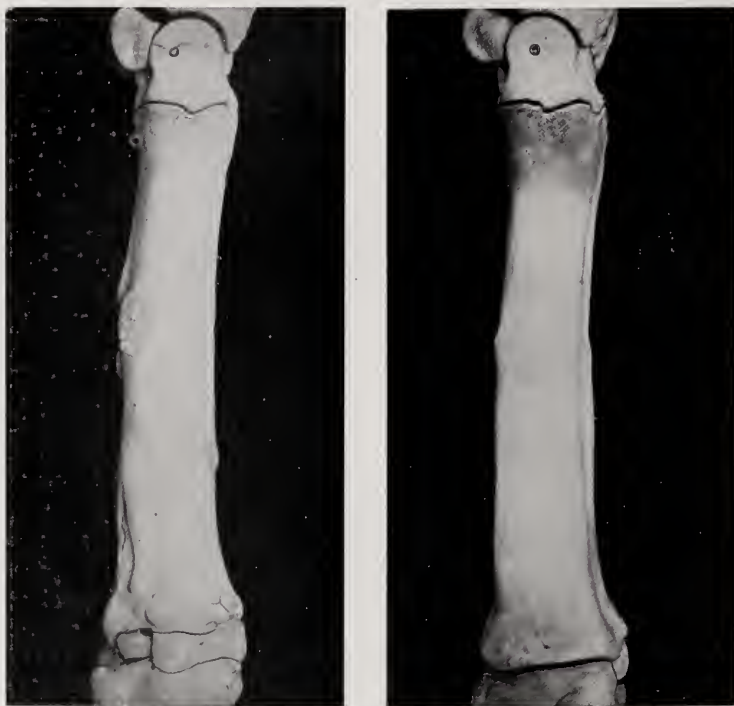


Fig. 7.—Long bones showing characteristic lesions.



Fig. 8.—Long bones showing characteristic lesions.

After the death of these two heifers their skeletons were examined for any unusual lesions. Characteristic swellings were found on the long bones of the extremities, especially the metacarpus and metatarsus. These lesions are well illustrated in the accompanying photographs (Figures 6, 7, and 8).

These lesions were present mainly on the diaphyses. They appeared as rounded excrescences varying in diameter from 1 to 3 centimeters or more. The surface was fairly smooth and one got the impression that abnormal growth of bone had taken place in the compact tissue; the periostium was not involved.

It should be added that these swellings were not painful on palpitation during life. The painful gait mentioned above was probably due to the swollen fetlock joints.

The microscopical changes in these bones will be described in a later paper.

In conclusion it should be mentioned that no specific changes could be found in any of the other organs of these animals.

SUMMARY AND CONCLUSIONS.

1. An experiment is described in which two heifers each received a daily dose of 5 grams sodium fluoride.

2. Two other heifers kept on the same basal ration, but receiving no sodium fluoride acted as controls and remained normal in every respect.

3. The two heifers receiving the fluoride showed a much poorer appetite and lost weight rapidly when compared with the controls. They showed other symptoms which suggested chronic poisoning. The one heifer died after calving and the other had to be destroyed.

4. Both heifers showed characteristic swellings on the metacarpus and metatarsus.

5. It is suggested that the condition of these animals was due to fluorine poisoning.

6. In another experiment cattle receiving rock phosphate showed somewhat similar changes, and it would seem likely that these changes too must be ascribed to the action of the fluorine in rock phosphate.

REFERENCES.

- BETHKE, R. M.; KICK, C. H.; EDGINGTON, B. H.; AND WILDER, O. H. (1930). The Effect of Feeding Sodium Fluoride and Rock Phosphate on Bone Development in Swine. *Amer. Soc. Anim. Prod. Proc.*, 1929, pp. 29-33.
- DU TOIT, P. J., AND MALAN, A. I. (1932). A Comparison of Phosphatic Supplements for the Prevention of Aphosphorosis. This Report.
- FORBES, E. B., AND OTHERS (1921). The Utilization of Calcium Compounds in Animal Nutrition. *Ohio Agric. Exp. Stn. Bull.* 347, p. 99.
- HUPKA AND GÖTZE (1931). Zur Forage der Schädlichkeit des Fluors beim Rinde. *Deuts. tierärztl. Wschr.*, 33, pp. 203-204.

- McCOLLUM, E. V.; SIMMONDS, N.; BECKER, J. E.; AND BUNTING, R. W. (1925). The Effect of Addition of Fluorine to the Diet of the Rat on the Quality of the Teeth. *Jnl. Biol. Chem.*, 63, pp. 553-562.
- McCLURE, F. J.; MITCHELL, H. H. (1930). The Effect of Calcium Fluoride and Phosphate Rock on the Calcium-retention of Young Growing Pigs. *Jnl. Agric. Res.*, Vol. 42, No. 6, 1931.
- REED, O. E., AND HUFFMAN, C. F. (1928). Dairy Cattle and Phosphorus in Ration. Lack of this Element causes Depraved Appetite in Animals. *Mich. Agric. Exp. Stn. Qlt. Bull.* 10, p. 156.
- REED, O. E., AND HUFFMAN, C. F. (1930). The Results of a Five-year Mineral Feeding Investigation with Dairy Cattle. *Mich. Agric. Exp. Stn. Tech. Bull.* 105, p. 63.
- SCHWYZER, F. (1903). The Pathology of Chronic Fluorine Poisoning. *Jnl. Med. Res.* 10, pp. 301-311.
- SCHWYZER, F. (1914). Einfluss chronische flurzufuhr auf dem Ali und Ca stoffwechsel.
- THEILER, A.; GREEN, H. H.; AND DU TOIT, P. J. (1927). Minimum Mineral Requirements of Cattle. *Jnl. Agric. Sc.*, Vol. 18, pp. 291-314.
- TAYLOR, G. E. (1929). Effect of Fluorine in Dairy Cattle Ration. Experimental Evidence indicates Fluorine Content of Raw Rock Phosphate is the Detrimental Factor. *Mich. Agric. Exp. Stn. Qlt. Bull.* 11, pp. 101-104.
- TOLLE, C., AND MAYNARD, L. A. (1929). Phosphatic Limestone and Other Rock Products as Mineral Supplements. *Amer. Soc. Anim. Prod. Proc.*, 1928, pp. 15-21.

Section VI.

Sex Physiology.

- J. QUINLAN AND I. P. MARAIS. Gland Grafting in Merino Sheep. Preliminary Observations on its Influence (c) on Castrated Sheep.
- J. QUINLAN, G. S. MARÉ AND L. L. ROUX. The Vitality of the Spermatozoon in the Genital Tract of the Merino Ewe, with special Reference to its Practical Application in Breeding.

Gland Grafting in Merino Sheep.

Preliminary Observations on its Influence: (c) on Castrated Sheep.

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and

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INTRODUCTION.

IN a previous paper Quinlan, Maré, and Roux (1930) published the results of their observations on the influence of gonad-grafting: (A) on body-development, wool-production, and progeny, and (B) on senility. That publication was confined to observations made on young rams which had been grafted with sections of testicle taken from an active adult ram, young ewes which had been grafted with the ovaries taken from active mature ewes, and a ram showing advanced senility, which was grafted on three occasions with grafts taken from active mature rams.

Since the above-mentioned publication appeared several workers have published their observations on gonad-grafting in sheep. [Gunn and Seddon (1930), Richter (1931), Miller 1931.]

Their experimental findings and those of previous workers, to whom reference was made in the first article, strongly support those published by us in 1930. Young rams do not appear to be influenced either in body-weight or wool-production by grafting, neither does the progeny of grafted rams show any beneficial influence transmitted by their sires. Most workers, however, agree with the observations made by us on the senile ram; that his general health improved considerably after grafting. Another suitable subject has since been submitted to the Voronoff operation with most encouraging results. The improvement, however, was again temporary. There seems to be little doubt that there is a temporary rejuvenation in senile rams after grafting. In the stage of onset of senility grafting appears to stimulate libido sexualis, but if the operation is postponed until the ram is already impotent the temporary improvement in general health does not appear to be accompanied by improved potency.

There is little doubt that much work has been done on rams to elucidate the problem of grafting so forcibly brought before sheep raising countries by Voronoff. However, the literature does not seem very rich in reference to work on sheep. The work done on other species of domestic animals appears to support our experimental evidence with senile rams [Hobday (1925), Schoupe (1928, Grabenko (1926), Krapivner (1926), Nikiforov (1926), Artemicév (1925)]. The beneficial results of grafting even in senile rams is of short duration, not longer than 6 to 8 months. In this connection Richter (1931) also supports the results published in South Africa. Repeated grafting does not appear to stimulate the general health in the same way after each operation. Successive graftings give less and less stimulus. (Quinlan, Maré, Roux, 1931.)

Gunn and Seddon (1930) and Richter (1931) have also studied the fate of the grafted tissue taken from the site of operation at intervals after grafting. Their histological findings agree with those published by Quinlan et al. and other workers, to whose publications reference was made in the previous paper, namely that the grafted tissue is rapidly absorbed, the surrounding tissue behaving as if the graft were a foreign body.

The temporary stimulus given to the general health by grafting does not seem to produce any permanent change in the testicles already showing senile atrophy, as indicated by Quinlan, Maré and Roux (1930).

Having carried out observations on young sheep of both sexes, and also on senile rams, it was decided to see what influence grafting sections of testicle might have on young castrated sheep. No reference can be found to this aspect of the problem in the literature available.

When the operation was considered it was realized that the only suitable situation for testicle grafts was the scrotal sac if the normal environmental temperature were to be maintained. Crew (1926), has indicated the detrimental effect of high temperature on the germinal epithelium of the testes. The cells of Sertoli and the interstitial cells are relatively much more resistant. Hammond and Asdell (1926), Hammond (1930), and Walton (1930) have shown that spermatozoa live longer in temperatures below that of the scrotum than in higher temperatures. It was considered, however, that if the interstitial cells and Sertoli's cells survived, as they do in the abdominal cryptorchid, the results of grafting, which appear to depend on other factors rather than spermatogenesis, would be shown. In the case of abdominal cryptorchidism, although the function of spermatogenesis is in abeyance, that of producing the internal secretion necessary for the development of the secondary sexual characteristics of the male is, as a rule, active.

It appeared unlikely that grafting into what remains of the fat-laden scrotum of the castrates would be a surgical success if the operation were delayed for any time after castration by actual removal of the gonads. It was consequently decided to transplant the sections of testicle in the abdominal wall in the region of the flank.

The sheep selected for the experiment were twelve high grade Merino wethers; Nos. (Controls) 23432, 23959, 24404, 24510, 24670, 24718; (Grafted) 22401, 23400, 23698, 24436, 24494, 24717. The selection was made so as to get as close uniformity as possible with regard to type, age, condition, body-weight, and wool. The sheep were all purchased from the same flock so that there was a relationship. By this method of selection it was hoped to eliminate, as far as possible, any tendency to individual variation, either of body-development or wool-production, due to inherent differences in individuals.

At the time of the operation all the sheep were about 18 to 21 months old. They had come from the Cape Province to Onderstepoort during the previous autumn and early winter. While at Onderstepoort they had all been used for the preparation of Bluetongue vaccine and had received the same food. Therefore, the selected sheep were as similar as it was possible to have them, and they had been in exactly the same environment and had the same daily ration for at least 8 months prior to being put into the experiment. Further, the sheep selected for operation and the control sheep were paired before the final choice was made.

THE OPERATION.

All twelve sheep were shorn prior to the commencement of the experiment. They were confined to the stable and put on to a ration of bran and succulent food (green lucerne) for a week. For 24 hours prior to the operation food was withheld, but water was allowed up to the 12th hour.

During the day previous to the operation the region of the right flank was shorn clean of wool and shaved. It was then painted over with tincture of iodine.

Two donors were used to supply the testicular tissue for the grafts. They were prepared for operation in the same manner as the experimental sheep. The scrotum was shaved and painted with tincture of iodine the day previous to the operation. The donors were carefully selected, being vigorous, two years old, Merino flock rams which were clinically normal.

Deep anaesthesia was used during the operation. Chloral hydrate in 10 per cent. solution with normal saline, was given intrajugularly. Each sheep, including the controls, received 45 c.c. of the solution. The average weight of the sheep to be grafted was 72.3 lb. and the weight of the controls was 73.5 lb. The donors which were heavier, averaged 115 lb., each received 50 c.c. of the chloral hydrate solution.

The grafts were removed with the strictest aseptic precautions. The donors were not killed; the grafts being removed while the testicles remained attached but exposed. Each section was cut from the surface of the testicle leaving the tunica vaginalis visceralis attached. The sections measured 6 cm. by 1.5 cm. by 1 cm. After removal from the donor they were immediately attached to the receptor which was already prepared.

The receptors, after anaesthesia was completed, were placed on the left side of the table and the site of operation washed off with ether. Sterile cloths were then clamped in position. An incision about 10 cm. long was made through the skin and fascia immediately in front of the *m. tensor fascialata*. Sterile gauze was attached to the lips of the cutaneous incision with forceps. Proceeding by blunt dissection two pockets were made; one between the *m. tensor fascialata* and the *m. obliquus externus*, and a second between the *m. transversus abdominis* and the *m. obliquus internus*. The surface of the muscles was prepared by scraping until it was oozing with blood. The grafts were attached by several sutures, using No. 00 catgut. The glandular surface of the section was placed in contact with prepared surface of the muscle. The graft was then covered over in the muscular pocket by suturing the two muscles together so that the whole graft was in close contact with a muscular wall. The muscle and fascia lying superficial to the graft were now sutured with a continuous catgut suture. The skin was closed with a series of interrupted silk sutures and the wound sealed with autosept.

The wound healed per primam intentionem; the stitches being removed on the 7th day.

The operations were performed on the 18th December and 20th December, 1929. On February 2nd one of the grafts sloughed away from sheep No. 24717, otherwise all the sections remained attached.

LOCAL RESULTS OF THE OPERATION.

There was a fairly well marked inflammatory swelling around the site of operation, which gradually subsided. It was then quite easy to feel the anterior graft by grasping the abdominal wall with the fingers and thumb. The posterior graft could also be felt by deep palpation. They remained unchanged in size for about two months, when a gradual diminution in size took place until, after a period of 5 to 8 months, they could no longer be palpated.

When the experiment was completed, at the end of twelve months, the site of the operation was carefully dissected out, but the only trace of foreign tissue which was found at the attachment of the sections of testicle, was a small firm thickening, about the size of a pea in some of the sheep. On cytological examination this was composed of connective tissue (Figs. 1 and 2). In other cases there was a capsule of fibrous tissue surrounding a centre of old granulation tissue already well advanced in a process of differentiation (Fig. 3). There was no cellular formation which would indicate that this tissue was of testicular origin. The testicular tissue had been completely absorbed.

TREATMENT DURING OBSERVATION AND GENERAL RESULTS FOLLOWING THE OPERATION.

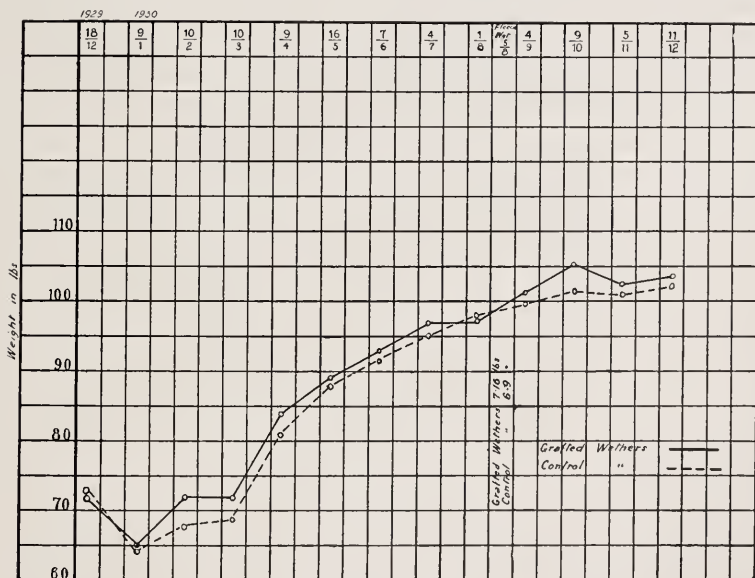
The experimental sheep were kept under observation for 12 months following the operation. All twelve, 6 grafted and 6 control sheep, were placed in a yard, 20 yards by 15 yards, where an open shelter was provided. They were under daily observation so as to see if there was any development of male tendencies. The ration

given consisted of one pound mealie meal, one-eighth ounce of salt, succulent food (green lucerne, green barley, or green oats), lucerne hay, and veld hay *ad lib.*, with a constant supply of fresh water. They were dosed at regular intervals with Government Wireworm Remedy so as to keep them as free as possible from intestinal parasites.

They were weighed at intervals of approximately a month, and were shorn on the 5th August following the operation; approximately $7\frac{1}{2}$ months growth of wool.

The weights of individual sheep are recorded below as well as the average group weights from month to month. The weight of wool shorn is also given (Tables I and II). The average weights are also compared in the form of a graph.

The following graph shows a comparison of the average weights taken at monthly intervals for twelve months following the operation:—



DISCUSSION.

It will be observed that the two groups of sheep were selected in such a way that they were as similar as possible with regard to breed, type, age, body-weight and wool-production. The control sheep were anaesthetised at the same time as the sheep were grafted, so that the operation was the only difference in treatment. After operation they were kept under identical conditions.

Just previous to the operation, 18.12.29, the average difference of weights of the two groups was 0.8 lb.; the control group being the heavier. During the 3 weeks following the operation, until 9.1.30, both groups lost weight at the same rate. After this time there was

TABLE I.
CONTROL WETHERS.

D.O.B. Nos.	18.12.29	9.1.30	10.2.30	10.3.30	9.4.30	16.5.30	7.6.30	4.7.30	1.8.30	Wgt. of Fleece, 5.8.30	4.9.30	9.10.30	5.11.30	11.12.30
23432	84 56 90 69 70 67	80.5	86	90	101	103	108	111.5	113.5	9.5	115	115.5	118	118
23959		47	43	46.5	56.5	63.5	67	68.5	71.5	5.5	73	74.5	74	70.5
24404		85	91.5	90	100	108	110	111	115.5	6.5	119	119	116.5	117
24510		63.5	68	66	76	82	85.5	89	92	7.5	88	93	92	96
24070		63.5	70.5	73	86.5	97.5	96	100	*	6.0	—	—	—	—
24718	67	50	44	48	65	71.5	81	90	95.5	6.5	104.5	105.5	106.5	112
Total.....	436	389.5	403.0	413.5	485	525.5	547.5	570.0	488.0	41.5	499.5	507.5	507.0	513.5
Average..	72.3	64.9	67.2	68.9	80.8	87.6	91.3	95.0	97.6	6.9	99.9	101.5	101.4	102.7

* Sheep sick, monthly weighing stopped.

TABLE II.
GRAFTED WETHERS.

D.O.B. Nos.	18.12.29	9.1.30	10.2.30	10.3.30	9.4.30	16.5.30	7.6.30	4.7.30	1.8.30	Wgt. of Fleece, 5.8.30	4.9.30	9.10.30	5.11.30	11.12.30
22401	71	63	69	60.5	77	81	86.5	91.5	95	9.5	92	97	92.5	96
23400	88	82.5	90.5	89.5	103.5	111	117	121	122.5	7.25	127	131	128	127
23698	69	65	74	77	89	96	100	106	106	8.25	105	114	115.5	115
24436	77	70.5	75.5	78	88.5	90	93	94.5	95	6.5	96	98	93	98
24494	63	55.5	60	64	70.5	79	77	81	81	6.75	*	85	81	82
24717	61	53.5	59	60.5	72	77	82	87.5	84	4.5	85	85	81	82
Total....	429	390.0	428.0	429.5	500.5	534	555.5	581.5	583.5	42.75	505	525	510	518
Average..	71.5	65.0	71.3	71.6	83.6	89	92.6	96.7	97.3	7.16	101	105	102	103.6

* Sheep sick, monthly weighing stopped.

an increase; the grafted sheep increasing more rapidly than the controls. There was an average increase of 4.1 lb. in favour of the grafted group on 10th February, 1930. This increase was reduced to 2.7 lb. a month later, on 10th March, 1930. The two curves, as shown in the graph, slowly approached the same average weight until August 1st, 1930, when they were almost identical. Following this date, except at one weighing, on the 9th October, 1930, the curve of the grafted group was always just above that of the controls until the termination of the experiment on the 11th December, 1930.

It would appear that the grafted sheep after an interval of 3 weeks following the operation had received some stimulus from the grafts. The stimulus was greatest during the first 3 months. During the following 5 months it gradually became less until at the end of 8 months it was no longer apparent.

The stimulus would appear to be developed during the life of the graft. It is greatest for a time after transplantation when the graft is largest, but decreases with the absorption of the testicular tissue.

The growth of wool did not appear to be noticeably stimulated; the average difference in fleece-weight after seven-and-a-half months growth being 0.26 lb. in favour of the grafted group.

Since the average difference in weight between the two groups after the 4th July, 1930, that is approximately $6\frac{1}{2}$ months after grafting, it was just a fraction above or a fraction below 1 lb., it would appear that the skeletal development was not influenced by grafting. It appears rather to be a stimulus to the general health with a consequent tendency to put on mutton.

The temperament did not appear to be influenced in any way. The grafted sheep behaved throughout the experiment as the control wethers.

CONCLUSIONS.

(1) Grafting sections of testicle into wethers has been successfully performed under general anaesthesia.

(2) Grafting does not appear to influence the temperament of wethers. They do not become masculine in their behaviour.

(3) Grafting appears to stimulate wethers so that they put on mutton somewhat more rapidly than control wethers.

(4) The grafted sheep and the controls again reached the same weight curve after a period of seven-and-a-half months, so that it appears the increase is due to mutton-production rather than to skeletal weight.

(5) The stimulus given to castrates by grafting is very transitory. It is greatest up to the end of the third month and afterwards gradually disappears until it is no longer evident after seven-and-a-half months.

(6) The increase in weight is not very marked; the greatest average difference being 4.1 lb. This was maintained only for a very short period.

(7) Grafts removed twelve months after operation showed no structural elements by which the tissue of their origin could be recognized.

LITERATURE.

- ARTEMICEV (1925). Ein Fall von Verjüngung eines Bockes. *Vet. Trujnik* (russ.) 3, 15. Cit. Richter.
- CREW, F. A. E. (1926). The Scrotum: A Temperature-regulating Mechanism. Verhandlungen des I. Internationalen Kongresses für Sexualforschung. Berlin, October, Marcus u. Weber. Berlin und Köln, 1927.
- GUNN, R. M. C., AND SEDDON, H. R. (1930). Testicular Grafts on Rams. *Aus. Vet. Jl.*, Vol. 6, No. 3, pp. 104-112 and No. 4, pp. 132-145.
- GRABENKO (1926). Zur Frage über die praktische Anwendung der Operationen nach Steinach und Voronoff bei landwirtschaftlichen Haustieren zur Verjüngung und zu therapeutischen Zwecken. *Vestn. Sovrem. Vet.* 9, 20. Cit. Richter.
- HAMMOND, J. (1930). The Effect of Temperature on the Survival in Vitro of Rabbit Spermatozoa Obtained from the Vagina. *Jl. Exp. Biol.*, Vol. 7, No. 2.
- HAMMOND, J., AND ASDELL, S. A. (1926). The Vitality of the Spermatozoa in the Male and Female Reproductive Tracts. *Br. Jl. Exp. Biol.*, Vol. 4, No. 2, pp. 155-185.
- HOBDAY, F. (1925). The Voronoff (Testicle Grafting) Rejuvenation Operation. *Vet. Jl.*, Vol. 82, pp. 37-39.
- KRAPIWNER (1926). Une expérience de rejeunissement chez le cheval. *Rev. Gén. Méd. Vét.*, Vol. 35, p. 101. Cit. Richter.
- MILLER, W. C. (1931). Rpt. Imp. Wool Conference, March, 1930. His Majesty's Stationery Office, London.
- NIKIFOROV (1926). Versuch einer Verjüngung eines Hengstes durch Transplantation von Hoden. *Vet. Trujnik* (russ.), 10.11.29. Cit. Richter.
- QUINLAN, J., MARE, G. S., AND ROUX, L. L. (1930). Gland Grafting in Merino Sheep. *16th Rpt. Dir. Vet. Ser. An. Ind.* U. of S.A.
- RICHTER, J. (1931). Experimentelle Untersuchungen über die Keimdrüsenüberpflanzung nach Voronoff bei Schaafböcken. *Archiv. für Tierernährung und Tierzucht*, Vol. 5, 3.
- SCHOUPPE, K. (1928). Über einige Verjüngungsversuche nach Voronoff bei Hunden. *W.T.M.*, Vol. 15, pp. 781-791.
- WALTON, A. (1930). The Effect of Temperature on the Survival in Vitro of Rabbit Spermatozoa obtained from the Vas Deferens. *Jl. Exp. Biol.*, Vol. 7, 2.

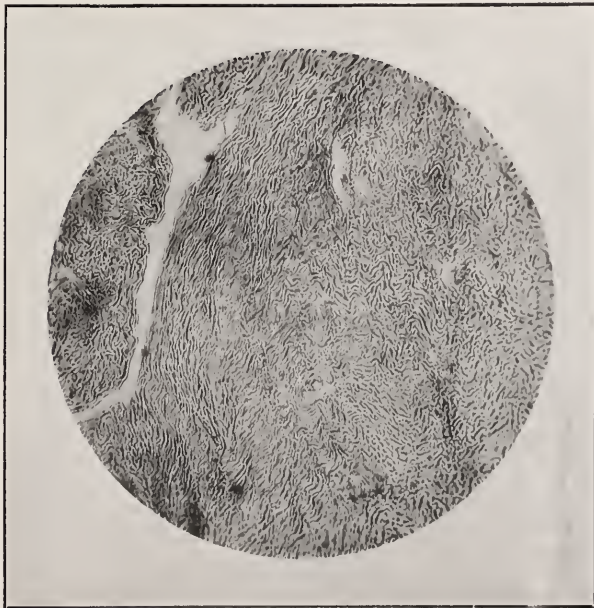


Fig. 1. Shows section of a transplant removed twelve months after operation.



Fig. 2. Shows section of a transplant removed twelve months after operation.

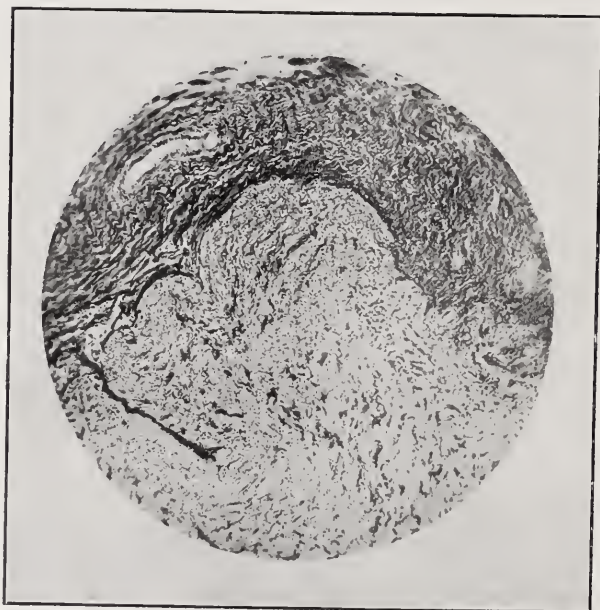


Fig. 3. Shows section of a transplant removed twelve months after operation.



Fig. 4. Control wethers at the time of operation.



Fig. 5. Grafted wethers at the time of operation.



Fig. 6. Control wethers, 26.11.30. The sick sheep, 24670, is second from the right.



Fig. 7. Grafted wethers, 26.11.30. The sick sheep, 24494, is not with the group.

The Vitality of the Spermatozoon in the Genital Tract of the Merino Ewe, with Special Reference to its Practical Application in Breeding.

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INTRODUCTION.

IN a previous paper Quinlan and Maré (1931) discussed the physiological changes in the ovary of the Merino sheep in South Africa and their practical application in breeding. The necessity for investigations into this problem was suggested to the authors on account of the high percentage of ewes which returned to service when the controlled service system of mating was adopted. Owing to the high price of proved stud sires in this country, and the fact that many farmers find it necessary to import Merino stud rams from Australia, it is desirable that a good stud ram should be used as freely as possible. Consequently the controlled service method of mating has been adopted by many of the foremost stud-breeders. This method of mating has been used for some years with the Government's stud-flock at the School of Agriculture, Middelburg, Cape Province. It was on this flock that Quinlan and Maré carried out their original observations which were published in the above-mentioned paper.

If a ram's energy and fertility were to be conserved as far as possible, it is desirable that only one mating with each ewe should be allowed. Under the usual conditions of mating in this country, where rams run with the ewes during the mating season, October to December, and April and May, an average of 50 ewes is allowed to each ram. Under the controlled service system 150 ewes or even more to each ram should be possible. It is, therefore, desirable that there should be as many conceptions as possible at the first service.

In the previous work Quinlan and Maré showed that the Merino ewe has a continuous ovarian cycle throughout the year, provided the grazing was good; the cycle recurred within 16 to 19 days as a rule, although cases were found on either side of this range.

The average duration of oestrus was about 40 hours; it may be as short as 24 hours and as long as 96 hours; while it was shown that ovulation occurred at about the 36th to the 40th hour of the oestrus period.

It was necessary to ascertain at what period during oestrus service would be followed by the greatest number of conceptions. This problem depended on two factors: (1) The vitality of the spermatozoon in the genital tract of the ewe, and (2) the vitality of the ovum after ovulation; that is, in reality, a time relation between the life of the ovum or ova and that of the spermatozoon.

During this study it was realized that spermatozoa, although motile, may yet be incapable of fertilization (Walton, 1926, Wolf, 1921, Hammond and Asdell, 1926, Knaus, 1931). On this account the vitality of the spermatozoa in the genitalia of the ewe as well as the practical aspect, namely, the time motile sperms in the genitalia are capable of fertilization, have been studied. The vitality of sperms in the male tract has not been studied during these experiments, but the authors are continuing with this work.

LITERATURE.

Although much work has been done on laboratory animals, especially the rabbit and the rat, the literature is not rich in references to work done on the vitality of spermatozoa in the sheep. The work of McKenzie and Phillips (1931) is the only reference available. These authors, working with Hampshire, Shropshire and Southdown ewes, which showed a duration of oestrus of 30.7, 26.3 and 24.0 hours respectively, had them mated; (1) during the first six hours of oestrus, (2) six to fourteen hours after the onset of oestrus, and (3) later than 14 hours after the onset of oestrus. The authors state: "When mated to rams of only fair fertility 70 per cent. of the ewes bred after the fourteenth hour of oestrus conceived, while only 35 per cent. of the ewes bred before that hour conceived. When a highly fertile ram was used the percentages were 100 and 66 respectively. Even highly fertile spermatozoa evidently meet with failure about one-third of the time when deposited in the early stages of oestrus, hence the importance of breeding in the latter part of oestrus in the ewe, or rather avoiding the early hours when hand-breeding is practised; that is when the use of the ram is limited."

A short review of the literature, relevant to the subject, on work which has been done on other animals will not be out of place.

The vitality of spermatozoa in the genitalia of the rabbit has been studied by Hammond and Asdell (1926). They conclude that sperms may retain their fertility in the male tract for a period of 38 days, but in the female tract only up to 30 hours. Walton, Hammond and Asdell (1928) have investigated the vitality of the spermatozoa of the rabbit outside the body, both those which were collected from the vagina after mating and those collected from the epididymis of the male immediately after killing. The semen collected from the vagina was, in favourable cases, capable of fertilizing after having been kept outside the body for a period of 66 hours, while that collected directly from the epididymis of the male was, in one case, fertile after 78 hours. Experiments were made by sending the semen by post, and out of 5 does inseminated, two days after sending, three produced litters. Yochem (1929)

studied the duration of life of spermatozoa in different portions of the female genital tract of 13 guinea-pigs and 14 rats after normal matings. Similar studies were made of the spermatozoa artificially injected into 11 guinea-pigs and 6 rats at oestrus and 23 guinea-pigs during the interoestrous period. It was found that there were motile sperms in the uterine horns and oviducts of the guinea-pigs 41 hours after mating, and in the rats 17 hours after mating. In the cases of insemination during oestrus, motility was maintained for 41.5 hours in the guinea-pigs and 12.5 hours in the rats. The duration of life of the sperm artificially injected during the interoestrous period was 36 hours in the guinea-pig. There was thus no physiological difference in the uterus at oestrus and inter-oestrus, which could be detected in the life of the spermatozoa. However, injections of the sperms of the rat into the guinea-pig survived but 4.5 hours, and guinea-pig sperm injected into the female rat but 11 hours. It thus appears that a non-species uterus has a marked effect on the vitality of spermatozoa.

Courrier (1922-23) has indicated the possibility of the sperms of the bat living throughout the winter in the female uterus and still being capable of fertilizing the ova in the spring. This may possibly be explained by the lower temperature during hibernation (Walton, 1930, Hammond, 1930, Crew, 1926). Redenz (1929) working on the bat reports that mating occurred in the autumn when the testicles of the male showed active spermatogenesis, but the sperm lay dormant in the female until the spring when ovulation occurred.

There is no doubt that the sperms of the domestic fowl survive many days in the female genitalia, but in this species the temperature of their new environment would differ little, if at all, as the testes of the male are abdominal. Anderson (1922) has done some observations on spermatozoa in the genitalia of the hen. He isolated hens for 24 days and then mated them with a vigorous cock. They were then destroyed at periods varying from 6 to 48 hours. All hens destroyed at the end of six and eight hour periods showed live sperms. None of the spermatozoa had progressed farther than one-third the length of the oviduct. He was unable to find any sperms, dead or alive, after a lapse of fifteen hours.

On the other hand, the short survival of the spermatozoa in the mouse and the guinea-pig has been pointed out by Sabotta (1895), Hoehne and Behne (1913, 1914).

It appears certain that the life of the spermatozoa in the female of domestic animals is short, since it would appear from the frequency distribution of the duration of pregnancy that the sperms cannot live during a normal interoestrous period. Further, it is rarely possible to fertilize a female by artificial insemination except during an oestrous period.

In woman the recent observations of Knaus (1931) indicate that the life of the sperms is of short duration. He has observed that in women with an intermenstrual period of more or less 28 days, that rupture of the follicle takes place about the 13th or 14th day, and that copulation is not followed by conception outside the 11th to the 17th day after the onset of menstruation. The observation of Knaus support those made by Siegel (1916), although the latter's dates for

physiological sterility in women differ from those of Knaus. This indicates that the life of the human sperm in the genitalia of women is, at any rate, confined to a week, and further that the extruded ovum is also available for fertilization only for a short time. Giles' (1919) remarks regarding the duration of viability of the human sperm are interesting. He states: "Spermatozoa in the vagina die within one hour after coitus; in the cervical canal they may be found in many cases as long as 2 to 5 days after coitus; in the fundus they are frequently found 24 hours after coitus, and occasionally after several days. Higher up, that is in the Fallopian tubes, their normal behaviour is unknown." Haussman and Hühner (1879) quoted by Giles, maintain the life of spermatozoa in the vagina is not longer than a few hours. In the cervical canal Hühner (1913) has shown that living sperms have been found after 15 to 24 hours only in 11·6 per cent. of cases; after 2 to 5 days in 20 per cent. of cases, and after 1 to 12 hours in 45·9 per cent. of cases. The same author's results of an examination for sperms in the uterus have shown living spermatozoa in 27 per cent. of cases after 1 to 12 hours; in 16·7 per cent. after 15 to 24 hours, and in 6·3 per cent. after 2 to 7 days.

Hirschfield, quoted by Bab (1908) found living sperms in the Fallopian tubes 14 to 16 hours after coitus and Baily and Miller (1911) state that they have seen them three and a half weeks after coitus.

Anderson (1922) has shown that the vitality of the sperm cell in the stallion, under laboratory conditions, is about six hours. The vitality is longer at low temperatures than towards body heat. Anderson's observations on the vitality of equine sperm cells *in vivo* are very limited. In one case no living sperms were found after a lapse of sixteen hours. In a second case vitality had ceased five hours after copulation, but he says this mare had a somewhat inflamed and congested uterus. In another case living and active sperms were present 7½ hours after mating. Hutschenreiter (1915) showed that sperms in the vagina of the mare were motionless inside five or six hours. Hammond (1931) has shown that coitus which occurred within three days from the termination of oestrus was followed by a greater number of pregnancies than when coitus occurred earlier during the heat period. In fact he found the frequency of pregnancy following coitus to be less when the earlier mating was allowed during the oestral period. He interprets this as evidence of the limited life of the spermatozoon in the genital tract of the mare. When coitus takes place early during oestrus the sperms have but a small chance of surviving until ovulation.

Wester (1921) has observed living spermatozoa in the cervix of the cow and goat up to 40 hours after copulation, but says this must be about the end-point of their vitality. He cannot agree that the spermatozoa may survive several days in the genital tract of the female awaiting the ovum. He has found that the sperms may have entirely disappeared from the female genital tract after 48 hours. In one case he found only dead sperms in the uterus and tubes after 63 hours. Renkert (1913) has also indicated that the life of the sperm cell in the genitalia of the cow is of short duration; after a stay of 5 to 6 hours in the vagina sperms were no longer motile.

Lewis (1911) has shown that the vitality of sperms in the genitalia of the sow varies considerably in individuals. Only in three cases could live sperms be found at a greater length of time than 20 hours after breeding. In two cases living cells were found 40 hours after copulation, and in one case after a lapse of 22½ hours. In 80 per cent. of nineteen sows killed for observation the sperm cells were found dead when a period of 16 hours or more had elapsed between copulation and the hour of slaughter.

EXPERIMENT 1.

To Ascertain the Duration of Vitality of the Spermatozoa and their Distribution in the Genitalia of the Merino Ewe.

In determining the length of life and the distribution of the sperms in the genitalia of the ewe after copulation three Merino rams, known to be highly fertile, were selected, namely, T.413, T.417 and W.31. These rams had been used in a previous experiment and their fertility records can be seen by consulting Table 2.

In the table T.413 is ram No. 1, T.417 is ram No. 2, and W.31 is ram No. 4.

The ewes selected for mating were mature sheep, in good condition, most of which had bred the previous season. They were tested twice daily for oestrus so that they were all in the early stage when service took place. Vasectomised teasers were used to pick out the sheep showing oestrus, and it is quite possible that all the ewes were served by the teaser once before they were mated to the fertile rams. Each sheep was served by at least two of the selected rams, 3 to 5 services being allowed altogether during an interval of 20 minutes or less.

After service the ewes were killed at intervals varying from 15 minutes to 48 hours. In some of the latter observations the ewes were not killed, but were anaesthetised while the genitalia were being examined for spermatozoa. The abdomen was opened through a pre-pubic mid-ventral incision and the different compartments of the genitalia clamped off with suitable forceps so as to prevent wandering of the spermatozoa.

The genital organs were at first removed and placed in moist cloths, but later, in many cases, they were left *in situ*. The different divisions were opened and fresh preparations were made on glass slides and immediately covered with a cover-slip. It was necessary to moisten the secretion from the Fallopian tubes in some cases with sterile 0.85 per cent. saline solution, otherwise the observations were made in the moisture of the natural secretion.

The preparations were immediately submitted to microscopic examination for living spermatozoa. Smears were also made for staining and later examination for morphological changes.

The shortcomings of such a method of examination are well recognized since it does not altogether simulate the environmental residence within the genitalia, but there was little chance of the sperms failing to survive the short interval between opening of the genitalia and microscopic examination. It is thought, therefore,

that when dead sperms were found on microscopic examination they were actually dead before the secretions containing them were removed from the genitalia. The examinations were done at room temperature which varied between 72° F. and 84° F. at the Grootfontein School of Agriculture, where the experimental observations were carried out.

The results are of sufficient interest to discuss them in some detail.

At an interval of 15 minutes after coitus living sperms were found in the vagina and the cervix in large numbers. No living sperms were found in the uterus, but a microscopic examination of stained preparations showed that a few had already reached as far as the pars indivisa. They were, however, very rare. Practically all the spermatozoa were actively motile. The divisions of the genitalia higher up did not contain any sperms.

At the sixth hour following coitus living sperms were present in the vagina, cervix, and uterus, reaching to the apices of the horns. Living sperms were seen in the uterine extremity of the tubes, showing that they had already reached the Fallopian tubes after such a short interval as six hours. In the stained preparations they were rare and difficult to find in the tubes in one case. Many of the spermatozoa in the vagina were non-motile in one case, but higher up in the genital tract 50 to 75 per cent. of them were motile.

Twelve hours following coitus sperms had penetrated to the tubes, but in this division of the genital tract they were relatively infrequent. The sperms in the vagina were mostly dead, over 75 per cent. being non-motile. A large percentage of those present in the cervix and the uterus were also dead. In another case examined after 12 hours interval following coitus living sperms were not seen in the tubes, although a few were seen in the stained preparation.

Fifteen hours following coitus live sperms were seen throughout the lower divisions of the tract, but were not seen in the tubes in one case, neither were sperms seen in the stained preparations made from the tubes in this case. Over 75 per cent. of sperms in the vagina were dead and others were sluggishly motile. Many non-motile sperms were also seen in the cervix and the uterus.

Eighteen hours following coitus live sperms were seen throughout all divisions of the genital tract in only one case. They were mostly immotile in the vagina; in one case all were non-motile; over 50 per cent. were motile in the cervix; 6 per cent. were motile in the uterus and uterine horns and 48 per cent. motile in the tubes in one case. Many of the non-motile sperms showed marked morphological changes.

Twenty-one hours following coitus live sperms were seen throughout the entire genital tract with the exception of the vagina, where all sperms seen were non-motile. Disintegrated remains of sperms were, however, plentiful. In two cases examined twenty-one hours following coitus, no sperms were found in the tubes either in the fresh or stained preparations.

Twenty-four hours following coitus live sperms have been seen in the cervix, uterus, uterine horns, and the tubes. In the tubes they appeared to be very infrequent and most difficult to find. In three cases all sperms in the vagina were non-motile and many showed disintegration. In a fourth case 36 per cent. of all complete sperms seen in the vagina were still sluggishly motile. In a fifth case less than 25 per cent. were motile in the vagina, but revealed only the slightest tail movement. There were many disintegrated specimens in this department of the genitalia. In the cervix a larger percentage of those present still showed life, although they were mostly sluggish of movement. Sperms in the uterus, uterine horns, and the tubes appeared to be very infrequent. In one case no sperms were seen in the tubes either in the fresh or stained preparations.

In one case examined after an interval of 27 hours sperms were seen throughout the entire tract, but they were all non-motile. Further up than the cervix they were very frequent. In another case examined at the same interval living sperms were seen in the cervix and pars indivisa only.

After an interval of 30 hours sperms were present and motile in the cervix. A few still seen in the vagina were non-motile. Many disintegrated remains were to be seen. Above the cervix sperms were not seen either in the fresh or stained preparations.

Following an interval of 36 hours after coitus sperms were seen in one case throughout the genital tract. They were fairly frequent, but sluggishly motile in the cervix. They were infrequent and non-motile in the vagina, uterine horns, and the tubes. The stained preparations showed morphological changes in most of the specimens.

After an interval of 39 hours living sperms were seen in the cervix, uterus and tubes, but not in the vagina.

After a forty-two hour interval following coitus 8 per cent. of sperms in the cervix were motile in one case. All others in this division were non-motile, many showing disintegration. Live sperms were still present in the uterine horns but they were very difficult to find; about 70 per cent. of those seen still showed sluggish motility. Rare sperms which were non-motile were seen in the vagina and the tubes. In a second case over 50 per cent. of living sperms were seen in the cervix, the uterus, and the tubes.

After an interval of 45 hours living sperms were seen in the cervix and uterus in one case. In another, living sperms were seen in the cervix only.

Following an interval of 48 hours after coitus in one case, no living sperms were seen in any compartment of the genital tract, although a few specimens showing morphological changes were present in the stained preparation from the cervix. In a second case living sperms were seen in the cervix, uterus, and uterine horns.

The following table gives a summary of the results obtained from an examination of the fresh and stained preparations taken from thirty-two sheep at different intervals following copulation.

VITALITY OF SPERMATOZOAN OF MERINO SHEEP.

TABLE 1.

Number of Sheep.	Interval after Service.	Number of Services.	Number of Rams Used.	Time taken to complete Services.	How Examined.	Divisions of the Genitalia Examined.				REMARKS.	
						Vagina.	Cervix.	Uterus, pars indivisa.	Uterus, Horns.		Fallopian Tubes.
O. 14	15 mins.	5	T. 413 × 3 W. 31 × 2	15 minutes	F.	XXXX	XXXX	—	—	Spermus very active. Infrequent in pars indivisa. No morphological changes.	
O. 26	6 hours	4	T. 413 × 2 W. 31 × 2	10 minutes	F.	XXXX	XXXX	XXX	XXXX	Spermus very active; only few seen in tubes. Some non-motile spermus show morphological changes and disintegration.	
O. 311	6 "	3	T. 413 × 1 W. 31 × 1 T. 417 × 1	4 "	F.	X	XXX	XXX	XXX	XX	Spermus actively motile. Not very infrequent in the tubes; many non-motile spermus show morphological change and disintegration.
O. 43	12 hours	5	T. 413 × 3 W. 31 × 2	15 minutes	F.	X	XXXX	XXX	XXX	O	Spermus active in cervix; infrequent higher up. Many disintegrated spermus in vagina.
O. 143	12 "	4	T. 417 × 2 T. 413 × 2	10 "	F.	X	XXXX	XXX	XXX	XX	Spermus active in cervix; infrequent higher up. Many dead and disintegrated spermus in vagina.
O. 259	12 "	3	T. 417 × 2 T. 413 × 1	5 "	F.	O	XX	XXX	XXX	XXXX	Spermus fairly active. Disintegrated remains in vagina. Many spermus show morphological changes.
O. 99	15 hours	4	T. 417 × 2 T. 413 × 2	15 minutes	F.	X	XXX	XX	XX	—	Spermus active in cervix. Some intact spermus show morphological change.
O. 239	15 "	3	T. 417 × 1 T. 413 × 1	10 "	F.	X	XXXX	XXX	XX	XX	Spermus in vagina, 2 per cent. sluggishly motile. Spermus actively motile in cervix. Very infrequent in tubes. Spermus in vagina show disintegration and lysis.
O. 60	18 hours	3	W. 31 × 1 T. 413 × 3	10 minutes	F.	Not done	Not done	Not done	Not done	Not done	Morphological changes.
O. 74	18 "	4	T. 417 × 2 T. 413 × 2	15 "	S.	X ¹	X ¹	X	X	—	Spermus active only in cervix.
O. 123	18 "	4	T. 417 × 2 T. 413 × 2	9 "	S.	X ¹	X ¹	X ¹	X ¹	—	Morphological changes.
O. 123	18 "	4	T. 417 × 2 T. 413 × 2	9 "	F.	O	XXX	X	X	XX	Spermus active only in cervix. Very infrequent in uterus and tubes. Morphological changes.
O.C. 9	18 "	4	T. 413 × 2 T. 417 × 2	20 "	S.	X	XXXX	X	X	O	Spermus active only in cervix. Very infrequent in uterus and tubes. Morphological changes.

(Continued.)

TABLE 1—(continued).

Number of Sheep.	Interval after Service.	Number of Services.	Number of Ram Used.	Time taken to complete Services.	How Examined.	Divisions of the Genitalia Examined.				REMARKS.	
						Vagina.	Cervix.	Uterus, pars interna.	Uterus, Horns.		Fallopian Tubes.
O. 142	21 hours	4	T. 413 × 2 T. 417 × 2	10 minutes	F. S.	O x ¹	xxxx x ¹	xx x ¹	x x ¹	x x ¹	Sperms active in cervix. Morphological changes.
O. 135	21 "	4	T. 417 × 2 T. 413 × 2	15 "	F. S.	O x ¹	xx x ¹	x x ¹	x x ¹	— —	Sperms active only in cervix. Many show morphological changes.
O.C. 5	21 "	4	T. 413 × 2 T. 417 × 2	20 "	F. S.	O x ¹	xxxx x ¹	xx x ¹	x x ¹	— —	Sperms active only in cervix. Many show morphological changes.
O. 69	24 hours	4	T. 417 × 2 T. 413 × 2	15 minutes	F. S.	xx x ¹	xxxx x ¹	O x ¹	O x ¹	— —	Sperms active only in cervix; very infrequent in uterine. Many show morphological changes.
O. 5	24 "	5	T. 413 × 3 T. 417 × 2	15 "	F. S.	x x ¹	xx x ¹	x x ¹	x x ¹	x x ¹	Sperms only sluggishly active. Very infrequent in uterine and tubes. Morphological changes.
O. 78	24 "	4	T. 413 × 2 T. 417 × 2	15 "	F. S.	O x ¹	xx x ¹	x x ¹	x x ¹	x x ¹	Sperms sluggishly motile. Morphological changes.
O. 97	24 "	4	T. 417 × 2 T. 413 × 2	15 "	F. S.	O x ¹	xx x ¹	x x ¹	x x ¹	O x ¹	Sperms sluggishly motile. Morphological changes.
O.C. 2	24 hours	4	T. 417 × 2 T. 413 × 2	20 minutes	F. S.	O x ¹	x x ¹	xxxx x ¹	xxxx x ¹	O x ¹	Sperms exceedingly infrequent in uterus and tubes. Disintegrated remains in vagina and cervix.
O. 112	27 hours	4	T. 413 × 2 T. 417 × 2	5 minutes	F. S.	O x ¹	O x ¹	O x ¹	O x ¹	O x ¹	All sperms seen non-motile. Disintegration.
O.C. 1	27 "	4	T. 413 × 2 T. 417 × 2	20 "	F. S.	O x ¹	xx x ¹	x x ¹	— —	— —	Sperms sluggishly motile. Morphological changes.
O. 156	30 hours	4	T. 417 × 2 T. 413 × 2	15 minutes	F. S.	O x ¹	x x ¹	— —	— —	— —	Small percentage of sperms sluggishly motile in the cervix. Disintegration.

[Continued.]

VITALITY OF SPERMATOZOAN OF MERINO SHEEP.

TABLE 1—(continued).

Number of Sheep.	Interval after Service.	Number of Services.	Number of Ram Used.	Time taken to complete Services.	How Examined.	Divisions of the Genitalia Examined.				REMARKS.	
						Vagina.	Cervix.	Uterus, pars Indivisa.	Uterus, Horns.		Fallopian Tubes.
O. 35	36 hours	5	T. 413 × 3 T. 417 × 2	15 minutes	F.	O	O	—	—	No living sperms seen. Disintegrated remains.	
					S.	x ¹	x ¹	x ¹	x ¹	Sperms infrequent.	
O. 124	36 "	4	T. 413 × 2 T. 417 × 2	15 "	F.	O	x	O	O	Sperms infrequent throughout.	
					S.	x ¹	x ¹	x	x ¹	x ¹	
O.C. 6	39 hours	4	T. 413 × 2 T. 417 × 2	20 minutes	F.	O	xxx	x ¹	x	x	Sperms active in cervix; infrequent higher up.
					S.	x ¹	x ¹	x ¹	x ¹	x ¹	Morphological changes.
O. 86	42 hours	4	T. 413 × 2 T. 417 × 2	10 minutes	F.	O	x	xx	xx	O	Sperms very infrequent throughout.
					S.	x ¹	x ¹	x ¹	x ¹	x ¹	
O.C. 7	42 "	4	T. 413 × 2 T. 417 × 2	20 "	F.	O	xxx	xxx	xxx	O	Sperms very infrequent throughout.
					S	x ¹	x ¹	x ¹	x ¹	x ¹	
O.C. 4	45 hours	4	T. 413 × 2 T. 417 × 2	15 minutes	F.	O	xxxx	xx	x	—	Sperms sluggishly motile; infrequent except in cervix. Morphological changes.
					S.	x ¹	x ¹	x ¹	x ¹	—	
O.C. 11	45 "	4	T. 413 × 2 T. 417 × 2	20 "	F.	O	x	O	O	—	Sperms very infrequent.
					S.	x ¹	x ¹	x ¹	x ¹	—	
O. 34	48 "	3	T. 413 × 1 T. 417 × 2	15 "	F.	O	O	—	—	—	Non-motile sperms very infrequent.
					S.	—	x ¹	—	—	—	
O.C. 3	48 "	4	T. 417 × 2 T. 413 × 2	20 "	F.	—	x	xxxx	xxxx	—	Sperms infrequent; only four seen in uterine horns, three being motile.
					S.	—	x ¹	x ¹	x ¹	—	

NOTE.—xxxx = 75-100 per cent. motile.
 xxx = 50-75 per cent. motile.
 xx = 25-50 per cent. motile.
 x = 10-25 per cent. motile.
 O = non-motile sperm only.

— = no sperm seen.
 x¹ = sperm seen in stained preparations.
 F. = Fresh preparations.
 S. = Stained preparations.

EXPERIMENT 2.

The purpose of this experiment was to ascertain the fertility established in Merino ewes when service is given at the onset of oestrus and at various definite periods after the onset: That is, the object was to determine the period of time during which motile sperms in the genitalia of the ewe are capable of fertilizing an available ovum.

EXPERIMENT 2A.

This portion of the experiment was conducted at the Veterinary Research Laboratory, Ermelo, Transvaal.

The physiographical conditions of the station well represent those of the eastern highveld of the Transvaal. The topography is undulating. The altitude is 5,690 feet above sea-level. The annual rainfall is in the vicinity of 27 inches, 90 per cent. of which falls during the summer months of October to April. The pastures contain a variety of grasses, among which rooigras (*Themeda triandra*) predominates; but, while the nutritive value of the pastures is relatively high during the early summer months, it is necessary to supplement the veld with oat grazing or with the feeding of concentrates during three winter months, June, July, and August.

Material Used.—The ewes and rams used for the experiment were taken from the station's Merino flock. The ages of the ewes ranged from 2 to 4 years; they were in good condition, their average weight being about 80 lb., and they were of the type which produces approximately 8 lb. of wool at 12 months' growth.

Two vigorous Merino flock rams were used to serve all the ewes in the experiment.

Procedure.—The ewes were tested for oestrus every hour daily by means of vasectomised teasers. Observations on oestrus were commenced on January 20th, 1931. The serving of the ewes for the experimental groups was completed on March 31st, 1931.

The hourly testing for oestrus was carried out as follows:—

Mondays to Fridays, 6 a.m. to 5.30 p.m.

Saturdays, 6 a.m. to 1 p.m.

Sundays, 9 a.m. (once only).

Ewes showing oestrus on Mondays to Saturdays at 6 a.m. and on Sundays at 9 a.m. were not served, but a record of such occurrences was kept.

Thirteen groups of sheep were made; each group consisted of 10 ewes. The selection for the groups was at random.

The following table indicates the periods during oestrus at which the various groups were served:—

TABLE 1. (EXPERIMENT 2A).

Group	1.—Ewes served at the onset of oestrus.						
"	2.	"	"	3	hours	after	onset of oestrus.
"	3.	"	"	6	"	"	"
"	4.	"	"	9	"	"	"
"	5.	"	"	12	"	"	"
"	6.	"	"	15	"	"	"
"	7.	"	"	18	"	"	"
"	8.	"	"	21	"	"	"
"	9.	"	"	24	"	"	"
"	10.	"	"	27	"	"	"
"	11.	"	"	30	"	"	"
"	12.	"	"	33	"	"	"
"	13.	"	"	36	"	"	"

All ewes were given one service by each of the two rams; the two services followed in quick succession.

In order to prevent over-taxing of the rams and thus possibly reducing their fertilizing powers, the daily services were distributed as much as possible.

Ewes indicating doubtful or indefinite oestrus were not accepted as showing oestrus until such time as they definitely "stood for" the teaser.

The groups were filled as evenly as possible during the course of the experiment.

Throughout the period of the experiment, the ewes were grazed on natural pasture. They maintained their condition remarkably well.

The rams received a small ration of maize and green feed during the earlier part of the experiment. It was found necessary to feed maize and bran during the latter half of the period of the experiment so as to maintain their condition.

All ewes were tested for oestrus for a period of at least 20 days after having been served. Ewes showing a recurrence of oestrus during the course of the experiment were again placed in one of the experimental groups. Towards the termination of the experiment, such ewes were served in order to test their fertility. Subsequent to the completion of the groups, testing for oestrus was continued once daily for 20 days. During this period, ewes showing a recurrence of oestrus were served in order to test their fertility. After this latter period, all ewes were run with the rams for a period of six weeks.

MATING SCHEDULES AND RESULTS.

The following tables, 2 to 14 (Experiment 2A) give full details of the thirteen groups of ewes which were served according to the plan of the experiment. The lambing results are also reflected.

Table 15 (Experiment 2A) contains extracts of the foregoing tables; this table reveals the degrees of fertility established in the various groups.

TABLE 2 (EXPERIMENT 2A).

GROUP 1.—EWES SERVED AT THE ONSET OF OESTRUS.

Ewe No.	Occurrence of Oestrus.		Service (double).		Date of Lambing.	Sex of Lambs.	Gestation Period (Days).
	Date.	Time.	Date.	Time.			
15261	24.1.31	11.30 a.m.	24.1.31	11.30 a.m.	24.6.31	M.	151
370	26.1.31	3.30 p.m.	26.1.31	3.30 p.m.	—	—	—
13124	2.2.31	9.30 a.m.	2.2.31	9.30 a.m.	5.7.31	M.	153
(395)*	7.2.31	9.0 a.m.	7.2.31	9.0 a.m.	—	—	—
21543	13.2.31	3.0 p.m.	13.2.31	3.0 p.m.	15.7.31	F.	152
(36)*	28.2.31	11.0 a.m.	28.2.31	11.0 a.m.	—	—	—
13	13.3.31	3.30 p.m.	13.3.31	3.30 p.m.	14.8.31	F.	154
6	13.3.31	4.0 p.m.	13.3.31	4.0 p.m.	9.8.31	F.	149
356	21.3.31	12.50 p.m.	21.3.31	12.50 p.m.	21.8.31	F.	153
80	27.3.31	4.45 p.m.	27.3.31	4.45 p.m.	22.8.31	M.	148

TABLE 3 (EXPERIMENT 2A).

GROUP 2.—EWES SERVED 3 HOURS AFTER THE ONSET OF OESTRUS.

25950	22.1.31	2.30 p.m.	22.1.31	5.30 p.m.	23.6.31	F.	152
21615	26.1.31	2.30 p.m.	26.1.31	5.30 p.m.	29.6.31	M.	154
15399	5.2.31	12.10 p.m.	5.2.31	3.10 p.m.	8.7.31	F.	153
19444	7.2.31	7.30 a.m.	7.2.31	10.30 a.m.	7.7.31	F.	150
18423	14.2.31	8.45 a.m.	14.2.31	11.45 a.m.	10.7.31	M.	146
15282	14.2.31	9.0 a.m.	14.2.31	12 noon.	16.7.31	F.	152
12111	4.3.31	4.0 p.m.	4.3.31	7.0 p.m.	1.8.31	F.	150
(18500)*	7.3.31	9.30 a.m.	7.3.31	12.30 p.m.	—	—	—
12106	11.3.31	4.40 p.m.	11.3.31	7.40 p.m.	10.8.31	F.	152
16086	30.3.31	9.15 a.m.	30.3.31	12.15 p.m.	30.8.31	M.	153

TABLE 4 (EXPERIMENT 2A).

GROUP 3.—EWES SERVED 6 HOURS AFTER THE ONSET OF OESTRUS.

21573	27.1.31	10.45 a.m.	27.1.31	4.45 p.m.	29.6.31	M.	153
25957	3.2.31	10.0 a.m.	3.2.31	4.0 p.m.	1.7.31	F.	148
52	6.2.31	8.20 a.m.	6.2.31	2.20 p.m.	4.7.31	M.	148
102	12.2.31	9.30 a.m.	12.2.31	3.30 p.m.	13.7.31	M.	151
15284	20.2.31	10.45 a.m.	20.2.31	4.45 p.m.	23.7.31	F.	153
1664	27.2.31	10.15 a.m.	27.2.31	4.15 p.m.	27.7.31	M.	150
16	11.3.31	3.0 p.m.	11.3.31	9.0 p.m.	9.8.31	F.	151
89	24.3.31	2.30 p.m.	24.3.31	8.30 p.m.	22.8.31	F.	151
13413	27.3.31	4.0 p.m.	27.3.31	10.0 p.m.	25.8.31	M.	151
19458	28.3.31	2.20 p.m.	28.3.31	8.20 p.m.	25.8.31	F.	150

TABLE 5 (EXPERIMENT 2A).

GROUP 4.—EWES SERVED 9 HOURS AFTER THE ONSET OF OESTRUS.

15264	2.2.31	9.0 a.m.	2.2.31	6.0 p.m.	5.7.31	M.	153
15377	3.2.31	9.15 a.m.	3.2.31	6.15 p.m.	3.7.31	F.	150
21537	6.2.31	8.20 a.m.	6.2.31	5.20 p.m.	9.7.31	F.	153
23	18.2.31	8.15 a.m.	18.2.31	5.15 p.m.	19.7.31	M.	151
22013	25.2.31	11.0 a.m.	25.2.31	9.0 p.m.	—	—	—
15149	27.2.31	8.45 a.m.	27.2.31	5.45 p.m.	27.7.31	F.	150
21495	4.3.31	11.0 a.m.	4.3.31	8.0 p.m.	7.8.31	F.	156
21590	16.3.31	12.15 p.m.	16.3.31	9.15 p.m.	12.8.31	M.	149
15342	27.3.31	10.20 a.m.	27.3.31	7.20 p.m.	Aborted	15.7.31	—
(69)*	27.3.31	11.20 a.m.	27.3.31	8.20 p.m.	—	—	—

* These ewes showed no recurrence of oestrus but they were subsequently found to be non-pregnant.

VITALITY OF SPERMATOZOAN OF MERINO SHEEP.

TABLE 6 (EXPERIMENT 2A).

GROUP 5.—EWES SERVED 12 HOURS AFTER THE ONSET OF OESTRUS.

Ewe No.	Occurrence of Oestrus.		Service (double).		Date of Lambing.	Sex of Lambs.	Gestation Period (Days).
	Date.	Time.	Date.	Time.			
22050	2.2.31	8.0 a.m.	2.2.31	8.0 p.m.	30.6.31	M.	148
(379)*	13.2.31	8.30 a.m.	13.2.31	8.30 p.m.	—	—	—
371	23.2.31	6.0 p.m.	24.2.31	6.0 a.m.	27.7.31	M.	153
90	23.2.31	5.0 p.m.	24.2.31	5.0 a.m.	24.7.31	F.	150
(395)*	23.2.31	5.15 p.m.	24.2.31	5.15 a.m.	—	—	—
19416	25.2.31	8.30 a.m.	25.2.31	8.30 p.m.	28.7.31	M.	153
19	4.3.31	9.0 a.m.	4.3.31	9.0 p.m.	2.8.31	F.	151
18443	16.3.31	9.15 a.m.	16.3.31	9.30 p.m.	11.8.31	F.	148
15418	20.3.31	10.0 a.m.	20.3.31	10.0 p.m.	17.8.31	F.	150
15277	24.3.31	9.0 a.m.	24.3.31	9.0 p.m.	—	—	—

TABLE 7 (EXPERIMENT 2A).

GROUP 6.—EWES SERVED 15 HOURS AFTER THE ONSET OF OESTRUS.

15304	3.2.31	5.0 p.m.	4.2.31	8.0 a.m.	6.7.31	F.	152
64	4.2.31	4.15 p.m.	5.2.31	7.15 a.m.	5.7.31	M.	150
15394	6.2.31	3.0 p.m.	7.2.31	6.0 a.m.	8.7.31	M.	151
44	9.2.31	3.20 p.m.	10.2.31	6.20 a.m.	Died	1.5.31	Pregnant.
21526	13.2.31	4.15 p.m.	14.2.31	7.15 a.m.	13.7.31	F.	149
15257	19.2.31	4.45 p.m.	20.2.31	7.45 a.m.	19.7.31	F.	149
22031	25.2.31	3.0 p.m.	26.2.31	6.0 a.m.	25.7.31	F.	149
15433	6.3.31	4.45 p.m.	7.3.31	7.45 a.m.	6.8.31	M.	152
26	11.3.31	4.40 p.m.	12.3.31	7.40 a.m.	—	—	—
15356	18.3.31	3.30 p.m.	19.3.31	6.30 a.m.	18.8.31	M.	152

TABLE 8 (EXPERIMENT 2A).

GROUP 7.—EWES SERVED 18 HOURS AFTER THE ONSET OF OESTRUS.

(81)*	30.1.31	2.45 p.m.	31.1.31	8.45 a.m.	—	—	—
15259	9.2.31	2.20 p.m.	10.2.31	8.20 a.m.	13.7.31	F.	153
22037	11.2.31	2.15 p.m.	12.2.31	8.15 a.m.	12.7.31	F.	150
373	24.2.31	12.30 p.m.	25.2.31	6.30 a.m.	24.7.31	M.	149
3	25.2.31	2.45 p.m.	26.2.31	8.45 a.m.	29.7.31	M.	153
88	11.3.31	3.0 p.m.	12.3.31	9.0 a.m.	8.8.31	M.	149
(42)*	13.3.31	12 noon	14.3.31	6.0 a.m.	—	—	—
15921	13.3.31	12.40 p.m.	14.3.31	6.40 a.m.	12.8.31	F.	151
28	20.3.31	3.15 p.m.	21.3.31	9.15 a.m.	17.8.31	F.	149
(21549)*	26.3.31	4.15 p.m.	27.3.31	10.15 a.m.	—	—	—

TABLE 9 (EXPERIMENT 2A).

GROUP 8.—EWES SERVED 21 HOURS AFTER THE ONSET OF OESTRUS.

(50)*	26.1.31	2.45 p.m.	27.1.31	11.45 a.m.	—	—	—
92	4.2.31	10.0 a.m.	5.2.31	7.0 a.m.	7.7.31	F.	152
95	5.2.31	10.45 a.m.	6.2.31	7.45 a.m.	8.7.31	F.	152
15315	6.2.31	10.30 a.m.	7.2.31	7.30 a.m.	9.7.31	M.	152
15441	17.2.31	11.20 a.m.	18.2.31	8.20 a.m.	21.7.31	M. twins	153
398	24.2.31	9.20 a.m.	25.2.31	6.20 a.m.	28.7.31	F.	153
18444	27.2.31	1.0 p.m.	28.2.31	10.0 a.m.	29.7.31	M.	151
351	4.3.31	3.45 p.m.	5.3.31	12.45 p.m.	2.8.31	F.	150
65	17.3.31	3.0 p.m.	18.3.31	12 noon.	15.8.31	M.	150
18449	20.3.31	12 noon	21.3.31	9.0 a.m.	20.8.31	M.	152

* These ewes showed no recurrence of oestrus but they were subsequently found to be non-pregnant.

TABLE 10 (EXPERIMENT 2A).

GROUP 9.—EWES SERVED 24 HOURS AFTER THE ONSET OF OESTRUS.

Ewe No.	Occurrence of Oestrus.		Service (double).		Date of Lambing.	Sex of Lambs.	Gestation Period (Days).
	Date.	Time.	Date.	Time.			
15387	29.1.31	11.0 a.m.	30.1.31	11.0 a.m.	1.7.31	F.	152
(18500)*	31.1.31	9.20 a.m.	1.2.31	9.20 a.m.	—	—	—
45	5.2.31	8.20 a.m.	6.2.31	8.20 a.m.	6.7.31	1 F. and 1 M.	150
13388	12.2.31	10.0 a.m.	13.2.31	10.0 a.m.	17.7.31	M.	154
18421	19.2.31	4.30 p.m.	20.2.31	4.40 p.m.	21.7.31	M.	151
51	23.2.31	4.0 p.m.	24.2.31	4.0 p.m.	25.7.31	F.	151
368	2.3.31	3.0 p.m.	3.3.31	3.0 p.m.	3.8.31	F.	153
369	2.3.31	4.30 p.m.	3.3.31	4.30 p.m.	2.8.31	M.	152
(15378)*	11.3.31	5.0 p.m.	12.3.31	5.15 p.m.	—	—	—
354	30.3.31	10.30 a.m.	31.3.31	10.30 a.m.	28.8.31	M.	150

TABLE 11 (EXPERIMENT 2A).

GROUP 10.—EWES SERVED 27 HOURS AFTER THE ONSET OF OESTRUS.

12364	2.2.31	9.30 a.m.	3.2.31	12.30 p.m.	6.7.31	M.	153
15434	4.2.31	2.45 p.m.	5.2.31	5.45 p.m.	4.7.31	M.	149
5070	6.2.31	11.0 a.m.	7.2.31	2.0 p.m.	10.7.31	M.	153
18517	12.2.31	2.45 p.m.	13.2.31	5.45 p.m.	13.7.31	F.	150
15	19.2.31	2.45 p.m.	20.2.31	5.45 p.m.	21.7.31	M.	151
25915	24.2.31	2.30 p.m.	25.2.31	5.30 p.m.	26.7.31	F.	151
15260	5.3.31	11.15 a.m.	6.3.31	2.15 p.m.	5.8.31	M.	152
(25925)*	17.3.31	10.15 a.m.	18.3.31	1.15 p.m.	—	—	—
75	26.3.31	8.20 a.m.	27.3.31	11.20 a.m.	22.8.31	F.	148
(83)*	26.3.31	5.0 p.m.	27.3.31	8.0 p.m.	—	—	—

TABLE 12 (EXPERIMENT 2A).

GROUP 11.—EWES SERVED 30 HOURS AFTER THE ONSET OF OESTRUS.

18526	21.1.31	10.30 a.m.	22.1.31	4.30 p.m.	20.6.31	F.	149
12336	5.2.31	8.30 a.m.	6.2.31	2.30 p.m.	8.7.31	F.	152
21666	5.2.31	10.0 a.m.	6.2.31	4.0 p.m.	6.7.31	F.	150
(376)*	11.2.31	10.0 a.m.	12.2.31	4.0 p.m.	—	—	—
21585	19.2.31	12 noon	20.2.31	6.0 p.m.	22.7.31	F.	152
29	13.3.31	12.15 p.m.	14.3.31	6.15 p.m.	15.8.31	M.	154
50	17.3.31	9.0 a.m.	18.3.31	3.0 p.m.	15.8.31	F.	150
21654	23.3.31	12.40 p.m.	24.3.31	6.40 p.m.	25.8.31	M.	154
15329	25.3.31	12 noon	26.3.31	6.0 p.m.	22.8.31	F.	149
15351	25.3.31	12.45 p.m.	26.3.31	6.45 p.m.	23.8.31	F.	150

TABLE 13 (EXPERIMENT 2A).

GROUP 12.—EWES SERVED 33 HOURS AFTER THE ONSET OF OESTRUS.

15358	4.2.31	9.0 a.m.	5.2.31	6.0 p.m.	6.7.31	F.	151
(15349)*	5.2.31	9.30 a.m.	6.2.31	6.30 p.m.	—	—	—
(21590)*	10.2.31	8.45 a.m.	11.2.31	5.45 p.m.	—	—	—
(10)*	2.3.31	12 noon	3.3.31	9.0 p.m.	—	—	—
(15936)*	9.3.31	1.0 p.m.	10.3.31	10.0 p.m.	—	—	—
32	10.3.31	11.30 a.m.	11.3.31	8.30 p.m.	10.8.31	M.	152
100	11.3.31	12.40 p.m.	12.3.31	9.40 p.m.	10.8.31	M.	151
21658	16.3.31	10.45 a.m.	17.3.31	7.45 p.m.	15.8.31	F.	151
13458	16.3.31	11.50 a.m.	17.3.31	8.50 p.m.	Died	11.6.31	Pregnant.
101	19.3.31	11.0 a.m.	20.3.31	8.0 p.m.	15.8.31	F.	148

* These ewes showed no recurrence of oestrus but they were subsequently found to be non-pregnant.

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TABLE 14 (EXPERIMENT 2A).

GROUP 13.—EWES SERVED 36 HOURS AFTER THE ONSET OF OESTRUS.

Ewe No.	Occurrence of Oestrus.		Service (double).		Date of Lambing.	Sex of Lambs.	Gestation Period (Days).
	Date.	Time.	Date.	Time.			
25961	9.2.31	9.30 a.m.	10.2.31	9.30 p.m.	7.7.31	F.	147
15448	9.2.31	10.0 a.m.	10.2.31	10.0 p.m.	9.7.31	M.	149
15426	11.2.31	6.45 a.m.	12.2.31	6.45 p.m.	10.7.31	M.	148
(27)*	26.2.31	8.45 a.m.	27.2.31	8.45 p.m.	—	—	—
(84)*	9.3.31	8.30 a.m.	10.3.31	8.30 p.m.	—	—	—
(25)*	11.3.31	8.20 a.m.	12.3.31	8.20 p.m.	—	—	—
13135	12.3.31	9.0 a.m.	13.3.31	9.0 p.m.	12.8.31	F.	152
(99)*	16.3.31	9.15 a.m.	17.3.31	9.15 p.m.	—	—	—
(33)*	19.3.31	11.0 a.m.	20.3.31	11.0 p.m.	—	—	—
25908	25.3.31	8.30 a.m.	26.3.31	8.30 p.m.	22.8.31	M.	149

* NOTE.—In all groups the bracketed numbers indicate that the ewes showed a recurrence of oestrus subsequent to mating.

The following table has been compiled from the data given in tables 2 to 14:—

TABLE 15 (EXPERIMENT 2A).

Group.	Time of Service.	No. of Ewes showing Recurrence of Oestrus.	Other Ewes not Fertilized.*	Total not Fertilized.	Percentage of Ewes Fertilized.
1	At onset of oestrus.....	2	1	3	70
2	3 hours after the onset of oestrus	1	0	1	90
3	6 " " " "	0	0	0	100
4	9 " " " "	1	1	2	80
5	12 " " " "	2	1	3	70
6	15 " " " "	0	1	1	90
7	18 " " " "	3	0	3	70
8	21 " " " "	1	0	1	90
9	24 " " " "	2	0	2	80
10	27 " " " "	2	0	2	80
11	30 " " " "	1	0	1	90
12	33 " " " "	4	0	4	60
13	36 " " " "	5	0	5	50
TOTALS.....		24	4	28	

* These ewes showed no recurrence of oestrus but they were subsequently found to be non-pregnant.

It must be emphasized that the ewes were kept under close observation during the gestation period in order to enable the detection of abortions. One abortion occurred; this took place during the latter stage of gestation and the cause was considered to have been due to crowding in a barn.

The service tests for fertility of ewes which showed a recurrence of oestrus subsequent to having been mated in the experimental groups, revealed that the great majority of these ewes were not sterile as lambs were obtained from the test services. However, in three of the groups certain ewes failed to conceive in spite of controlled serving and having been run with the rams for six weeks. If these ewes are to be considered as having been sterile, the percentages of fertility in the groups affected would be as follows:—

Table 15, Experiment 2A—Group 2—100 per cent.

Table 15, Experiment 2A—Group 7—77·8 per cent.

Table 15, Experiment 2A—Group 9—100 per cent.

From the above table 15 it is apparent that fertility is established to a satisfactory degree in Groups 1 to 11, although in these groups the fertility ranges between 70 and 100 per cent. The results in these groups do not reveal a definite correlation between certain degrees of established fertility and the times of service subsequent to the commencement of oestrus.

The fall to 60 per cent. in the 33rd hour period appears to be of some significance, while a further fall to 50 per cent. in Group 13 is of great interest and definitely indicates decreased fertility during the later stages of oestrus.

It is of interest to note that some of the ewes which showed oestrus after having been mated in the experimental groups, were served in groups different to their original groups, while two ewes were mated in their original groups.

The following table indicates the results of such matings:—

TABLE 16 (EXPERIMENT 2A).

Ewe No.	First Mating.	Result.	Second Mating.	Result.	Third Mating.	Result.
395	At onset oestrus	Not fertilized	12 hours after onset of oestrus	Not fertilized	12 hours after onset of oestrus	Not fertilized.
18500	24 hours after onset of oestrus	„	3 hours after onset of oestrus	„		
50	21 hours after onset of oestrus	„	30 hours after onset of oestrus	Fertilized		
376	30 hours after onset of oestrus	„	30 hours after onset of oestrus	„		
21590	33 hours after oestrus	„	9 hours after onset of oestrus	„		

In connection with the above table it must be pointed out that the ewes which showed a recurrence of oestrus after having been mated in any of the experimental groups and which were then mated in the same group, were, in the latter case, not considered as additions to those groups.

Table 16 indicates to a small extent the individual differences that may exist.

Ewe No. 18500 (Tables 3 and 16, Experiment 2A) did not lamb, although she was run with the rams for six weeks after the termination of hand-serving. This ewe is, therefore, in all probability, sterile.

Ewe No. 395 (Tables 6 and 16, Experiment 2A) lambed; she was fertilized when run with the rams.

Ewes Nos. 50 (Tables 12 and 16, Experiment 2A) and 21590 (Tables 5 and 16, Experiment 2A) were fertilized at the second mating when they were served in groups other than their original groups.

The following table indicates the intensity of the serving of ewes demanded from the two rams.

Services exceeding two per diem have been included; the dates upon which less than three services were given by the rams have not been added, as there is no likelihood of the rams having been subjected to undue strain.

The last column indicates whether or not ewes served on the particular date subsequently showed oestrus; the hours at which these ewes were served are in *italic*.

TABLE 17 (EXPERIMENT 2A).

Date.	No. of Services.			Times of Services.		Returns.
	a.m.	p.m.	Total.	a.m.	p.m.	
2.2.31	1	2	3	9.30	6.0, 8.0	0
3.2.31	0	3	3	—	12.30, 4.0, 6.15	0
5.2.31	2	3	5	7.0, 7.15	3.10, 5.45, 6.0	0
6.2.31	2	5	7	7.45, 8.20	2.20, 3.30, 4.0, 5.20, 6.30, 7.54	1
7.2.31	4	1	5	6.0, 7.30, 9.0, 10.30	2.0	1
10.2.31	2	2	4	6.20, 8.20	9.30, 10.0	0
12.2.31	1	3	4	8.15	3.30, 4.0, 6.45	1
13.2.31	1	3	4	10.0	2.45, 3.0, 8.30	1
14.2.31	3	0	3	7.15, 11.45, 12.0	—	0
18.2.31	2	1	3	8.20, 8.45	5.15	0
20.2.31	1	4	5	7.45	4.30, 4.45, 5.45, 6.0	0
24.2.31	0	4	4	—	4.0, 5.0, 5.15, 6.0	0
25.2.31	2	3	5	6.20, 6.30	5.30, 8.30, 9.0	0
27.2.31	0	3	3	—	4.15, 5.45, 8.45	1
28.2.31	2	1	3	10.0, 11.0	3.30	1
3.3.31	0	3	3	—	3.0, 4.30, 9.0	1
4.3.31	0	3	3	—	7.0, 8.0, 9.0	0
11.3.31	0	3	3	—	7.40, 8.30, 9.0	0
12.3.31	2	4	6	7.40, 9.0	5.0, 8.20, 9.20, 9.40	3
13.3.31	0	3	3	—	3.30, 4.0, 9.0	0
14.3.31	2	1	3	6.0, 6.40	6.15	1
17.3.31	0	3	3	—	7.45, 8.50, 9.15	1
18.3.31	1	2	3	12.0	1.15, 3.0	1
20.3.31	0	3	3	—	8.0, 10.0, 11.0	1
21.3.31	2	1	3	9.0, 9.15	12.50	0
24.3.31	0	3	3	—	6.40, 8.30, 9.0	0
26.3.31	0	3	3	—	6.0, 6.45, 8.30	0
27.3.31	3	5	8	9.0, 10.15, 11.20	4.45, 7.20, 8.0, 8.20, 10.0	3
28.3.31	2	2	4	8.30, 10.30	1.15, 2.20	0

NOTE.—The above table includes 17 out of a total of 24 of the "returns" in all the groups.

The following table incorporates all services and further indicates the distribution of the services given by each of the two rams throughout the entire course of the experiment:—

TABLE 18 (EXPERIMENT 2A).

No. of services given per diem	0	1	2	3	4	5	6	7	8
No. of days on which such services were given.....	21	9	8	18	5	4	1	1	1

It is seen that on 21 days during the course of the experiment the rams did not serve; also the rams gave one service on each of nine days, two services on each of eight days, etc.

The total number of services given by each ram was 140 during the course of 68 days. Throughout this period the rams maintained vigorous condition and upon no occasion did they show reluctance or indifference when brought to the ewes.

A study of the above tables (Nos. 17 and 18, Experiment 2A) will indicate that in no case can the severity of service by the two rams be the cause of the failure of fertilization.

The following data which are really irrelevant to the object of these experiments became available during the course of this work, and they are published here for comparison with similar data, which were obtained at the Grootfontein School of Agriculture, and previously published by the two authors (Quinlan and Maré, 1931). It is considered that these observations are not out of place here as the climatic conditions prevailing at the Ermelo and Grootfontein stations are entirely different.

THE OESTROUS CYCLE IN MERINO SHEEP.

The following table has been compiled from the data taken during the course of the experiment and 20 days after its termination. During the latter period certain of the ewes were observed for oestrus and were served in order to test their fertility.

TABLE 19 (EXPERIMENT 2A).

<i>Period in Days.</i>	<i>Oestrous Cycle, Frequency.</i>	<i>Percentage of Total.</i>
9	1	0.6
12	1	0.6
14	1	0.6
15	2	1.1
16	40	22.1
17	85	47.0
18	41	22.6
19	5	2.8
20	1	0.6
21	1	0.6
33	1	0.6
34	1	0.6
35	1	0.6

NOTE.—Successive oestrous cycles were included in compiling the above table.

The mode is seen to be 17 days with an almost equal distribution between 16 and 18 days.

The following table indicates the number of occurrences of oestrus which commenced during the periods of time indicated.

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TABLE 20 (EXPERIMENT 2A).

6 a.m. to 12 noon.	12 noon to 5.30 p.m.	5.30 p.m. to 6 a.m.
72	58	208

It is computed that in 61.5 per cent. of cases oestrus commenced between 5.30 p.m. and 6 a.m.

THE CONSISTENCY AND VARIATION OF SUCCESSIVE OESTROUS CYCLES IN MERINO SHEEP.

No. of cases in which no variation occurred	29
No. of cases in which a variation of one day occurred	29
No. of cases in which a variation of two days occurred	6

Hence in 29 cases the immediate subsequent oestrous cycle was as long as the preceding cycle; in 29 cases the immediate subsequent oestrous cycle varied by one day as compared with the preceding cycle, and only in six instances was this variation two days.

TABLE 21 (EXPERIMENT 2A).

MARKED ABNORMALITIES OF SUCCESSIVE OESTROUS CYCLES.

(The following are not included in the above statement of consistency and variation of oestrous cycles.)

	<i>First.</i>	<i>Second.</i>	<i>Third.</i>	<i>Fourth.</i>
	<i>Days.</i>	<i>Days.</i>	<i>Days.</i>	<i>Days.</i>
Ewe No. 50.....	17	33	—	—
Ewe No. 80.....	17	19	14	—
Ewe No. 378.....	21	16	16	17
Ewe No. 394*.....	9	12	—	—
Ewe No. 15328.....	16	35	—	—
Ewe No. 15360.....	17	34	—	—

* Ewe No. 394 was mated 3 hours after the second occurrence of oestrus and oestrus did not recur during a subsequent testing period of 23 days. However, the ewe was seen to be standing for the ram 39 days subsequent to the last day of testing for oestrus. This ewe was not included in the experiment.

The following table concerning the gestation period has been compiled from data given in Tables 2 to 14 (Experiment 2A):—

TABLE 22 (EXPERIMENT 2A).

THE GESTATION PERIOD IN MERINO SHEEP.

<i>Frequency.</i>	<i>Gestation Period in Days.</i>
1.....	146
1.....	147
8.....	148
13.....	149
18.....	150
17.....	151
18.....	152
17.....	153
5.....	154
0.....	155
1.....	156

NOTE.—Two pairs of twins were born. The duration of pregnancy of each pair of twins was taken as one gestation period.

From the above table it is seen that the gestation period ranges from 146 to 156 days and the modes are 150 and 152 days. In 84 per cent. of the cases the gestation period ranged between 149 and 153 days. The mean gestation period has been computed to be 150.9 days.

The following table has been compiled from data given in tables 2 to 14. The purpose of the table is to determine whether a relationship exists between the sex of lambs and the duration of pregnancy.

TABLE 23 (EXPERIMENT 2A).

<i>Gestation Period in Days.</i>	<i>Frequencies.</i>	
	<i>Males.</i>	<i>Females.</i>
146.....	1	0
147.....	0	1
148.....	4	4
149.....	6	7
150.....	5	14
151.....	9	8
152.....	7	11
153.....	11	7
154.....	4	1
155.....	0	0
156.....	0	1
TOTALS.....	47	54

NOTE.—In that unlike sexes occurred in one pair of twins, the gestation periods of the two pairs of twins were considered as being four periods.

From the above table it will be seen that the mode in the case of the males and females is 153 days and 150 days respectively. The arithmetical average or mean was computed to be 151.15 days and 150.79 days in the case of the males and females respectively.

The information available indicates that there is no significant difference between the duration of gestation in the case of male and female Merino lambs.

EXPERIMENT 2B.

This portion of the experiment was carried out at the Grootfontein School of Agriculture, Middelburg, Cape Province. The average annual rainfall at the School, based on records for 19 years, is 12.52 inches, of which 75 per cent. falls during the months of November to April. The altitude is 4,095 feet above sea-level. The grazing consists of typical Karroo bushes. The farming practice is to rely solely on veld grazing unless severe droughts occur. Normally no supplementary feeding is required.

The rams and ewes used in the experiment were taken from the Merino flock maintained at the School. The ewes were of an average type weighing approximately 75-80 lb., and producing about 9-10 lb. of wool at 12 months' growth. The ages of these sheep ranged from 2 to 4 years. During the first part of the experiment only two tested rams were used but subsequently the services of another proved sire were requisitioned.

Testing for oestrus was carried out daily from 6 a.m. to 6 p.m. at intervals of one hour. Vasectomised teasers were used to find the ewes. In order not to handle the whole flock continuously and to avoid repeated driving backwards and forwards to pens the following system was adopted. The teasing of the general flock was started 17 days prior to the commencement of the experiment and continued throughout the period that the work lasted. This was done once a day at 6 a.m. Ewes showing oestrus received a distinctive mark. When the work actually started those ewes which had shown oestrus 17 days previously were separated from the general flock and brought in from the veld to the sheds. This batch was then teased hourly as stated above. The process was repeated daily, and in this way only ewes due to show oestrus within a short while were handled at hourly intervals. The system was an unqualified success, as only a few individuals failed to fulfil expectations of the attendant in regard to occurrence of oestrus. The work stretched over the following periods:—

1st period, February 13th to March 10th, when 48 ewes were served.

2nd period, May 2nd to May 10th, when 31 ewes were served.

3rd period, May 18th to June 23rd, when 71 ewes were served.

These 150 ewes were placed in 15 groups of 10 each. The selection for the groups was at random.

The following table indicates the period during oestrus at which service took place:—

TABLE 1 (EXPERIMENT 2B).

Group	1 ewes served at the onset of oestrus.			
"	2	"	3	hours after the onset of oestrus.
"	3	"	6	"
"	4	"	9	"
"	5	"	12	"
"	6	"	15	"
"	7	"	18	"
"	8	"	21	"
"	9	"	24	"
"	10	"	27	"
"	11	"	30	"
"	12	"	33	"
"	13	"	36	"
"	14	"	39	"
"	15	"	42	"

All ewes were given four services by two or more of the rams used. The services followed in quick succession.

Care was taken to distribute the services of the rams as much as possible in order to prevent overtaxing them and possibly reducing their powers of fertilization.

Ewes were not accepted as showing oestrus until such time as they definitely "stood for" the teaser. Alternate groups were filled as evenly as possible during the course of the experiment. The rams were stall fed on lucerne hay *ad lib.*, supplemented with adequate quantities of whole oats and mealies. They retained their vigour and eagerness to serve ewes throughout. The ewes grazed on natural pasture during the period of the experiment. They maintained their condition well.

The flock of served ewes was kept under close supervision and teased daily for at least 20 days after service. Eleven of the ewes which showed a recurrence of oestrus were again placed in one of the experimental groups (Table 17, Experiment 2b). Ewes which did not show a recurrence of oestrus were killed, as is indicated in tables 2 to 16 (Experiment 2b). The animals were kept under very close observation prior to slaughtering in order to enable the detection of abortions. No abortions occurred.

In tables 2 to 16 (Experiment 2b), details are given of the 15 groups of ewes which were served according to the plan of the experiment. The degrees of fertility are revealed.

TABLE 2 (EXPERIMENT 2b).

GROUP 1.—EWES SERVED AT ONSET OF OESTRUS.

Ewe No.	Occurrence of Oestrus.		Service 4 Times.		Recurrence of Oestrus.	Date Killed.	Result of Mating.
	Date.	Time.	Date.	Time.			
O. 79	13.2.31	11 a.m.	13.2.31	11 a.m.	—	24.3.31	*
O. 91	13.2.31	4 p.m.	13.2.31	4 p.m.	—	23.3.31	*
O. 141	16.2.31	8 p.m.	16.2.31	8 p.m.	—	25.3.31	*
O. 158	3.3.31	1 p.m.	3.3.31	1 p.m.	21.3.31	—	—
O. 126	4.3.31	2 p.m.	4.3.31	2 p.m.	—	22.4.31	*
O. 161	5.3.31	2 p.m.	5.3.31	2 p.m.	—	27.4.31	*
O. 157	2.5.31	3 p.m.	2.5.31	3 p.m.	—	23.7.31	*
MBA 55	2.5.31	5 p.m.	2.5.31	5 p.m.	—	23.7.31	*
G. 116	7.5.31	2 p.m.	7.5.31	2 p.m.	—	29.7.31	*
O. 115	19.5.31	1 p.m.	19.5.31	1 p.m.	—	10.8.31	*

TABLE 3 (EXPERIMENT 2b).

GROUP 2.—EWES SERVED 3 HOURS AFTER THE ONSET OF OESTRUS.

191	18.5.31	2 p.m.	18.5.31	5 p.m.	—	10.8.31	—
W. 231	22.5.31	11 a.m.	22.5.31	2 p.m.	—	10.9.31	*
O. 181	23.5.31	11 a.m.	23.5.31	2 p.m.	—	10.9.31	*
G. 130	24.5.31	2 p.m.	24.5.31	5 p.m.	—	10.9.31	*
O. 188	30.5.31	7 a.m.	30.5.31	10 a.m.	—	4.9.31	—
MX 23	4.6.31	2 p.m.	4.6.31	5 p.m.	9.7.31	—	—
O. 118	8.6.31	2 p.m.	8.6.31	5 p.m.	—	1.9.31	*
197	12.6.31	12 noon	12.6.31	3 p.m.	—	31.8.31	*
O. 106	18.6.31	1 p.m.	18.6.31	4 p.m.	7.7.31	—	—
MBA	19.6.31	10 a.m.	19.6.31	1 p.m.	8.7.31	—	—

N.B.—See Table 3a Expt. 2b in Appendix.

TABLE 4 (EXPERIMENT 2b).

GROUP 3.—EWES SERVED 6 HOURS AFTER THE ONSET OF OESTRUS.

O. 163	13.2.31	8 a.m.	13.2.31	2 p.m.	—	24.3.31	*
O. 111	13.2.31	9 a.m.	13.2.31	3 p.m.	—	20.3.31	*
O. 147	28.2.31	10 a.m.	28.2.31	4 p.m.	19.3.31	—	—
O. 76	1.3.31	12 noon	1.3.31	6 p.m.	—	15.4.31	*
O. 150	2.3.31	1 p.m.	2.3.31	7 p.m.	—	20.4.31	*
O. 105	3.3.31	1 p.m.	3.3.31	7 p.m.	22.3.31	—	—
O. 172	3.5.31	11 a.m.	3.5.31	5 p.m.	—	23.7.31	*
MBA 26	4.5.31	8 a.m.	4.5.31	2 p.m.	—	24.7.31	*
O. 63	10.5.31	9 a.m.	10.5.31	3 p.m.	—	6.8.31	*
W. 274	20.5.31	11 a.m.	20.5.31	5 p.m.	—	13.8.31	*

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TABLE 5 (EXPERIMENT 2B).

GROUP 4.—EWES SERVED 9 HOURS AFTER THE ONSET OF OESTRUS.

Ewe No.	Occurrence of Oestrus.		Service 4 Times.		Recur- rence of Oestrus.	Date Killed.	Result of Mating.
	Date.	Time.	Date.	Time.			
2399	24.5.31	9 a.m.	24.5.31	6 p.m.	—	9.9.31	*
4675	28.5.31	10 a.m.	28.5.31	7 p.m.	—	7.9.31	*
O. 94	29.5.31	9 a.m.	29.5.31	6 p.m.	—	7.9.31	*
175	30.5.31	7 a.m.	30.5.31	4 p.m.	—	4.9.31	*
O. 222	11.6.31	9 a.m.	11.6.31	6 p.m.	—	31.8.31	*
E. 100	14.6.31	7 a.m.	14.6.31	4 p.m.	—	27.8.31	*
O. 190	15.6.31	11 a.m.	15.6.31	8 p.m.	—	27.8.31	*
O. 164	17.6.31	10 a.m.	17.6.31	7 p.m.	—	25.8.31	*
O. 82	18.6.31	8 a.m.	18.6.31	5 p.m.	—	24.8.31	*
O. 207	19.6.31	7 a.m.	19.6.31	4 p.m.	—	24.8.31	*

TABLE 6 (EXPERIMENT 2B).

GROUP 5.—EWES SERVED 12 HOURS AFTER THE ONSET OF OESTRUS.

O. 106	23.2.31	7 a.m.	23.2.31	7 p.m.	12.3.31	—	—
O. 65	25.2.31	7 a.m.	25.2.31	7 p.m.	—	1.4.31	*
O. 137	25.2.31	6 p.m.	26.2.31	6 a.m.	—	7.4.31	*
O. 121	1.3.31	6 p.m.	2.3.31	6 a.m.	—	16.4.31	*
O. 72	2.3.31	8 a.m.	2.3.31	8 p.m.	—	27.4.31	*
O. 67	3.3.31	7 p.m.	4.3.31	7 a.m.	—	21.4.31	*
G. 119	6.5.31	7 a.m.	6.5.31	7 p.m.	—	28.7.31	—
O. 147	8.5.31	7 a.m.	8.5.31	7 p.m.	—	4.8.31	*
O. 167	20.5.31	6 p.m.	21.5.31	6 a.m.	—	13.8.31	*
O. 177	21.5.31	6 p.m.	22.5.31	6 a.m.	—	17.8.31	*

TABLE 7 (EXPERIMENT 2B).

GROUP 6.—EWES SERVED 15 HOURS AFTER THE ONSET OF OESTRUS.

O. 139	20.5.31	6 p.m.	21.5.31	9 a.m.	—	17.8.31	*
O. 98	23.5.31	5 p.m.	24.5.31	8 a.m.	—	10.9.31	*
O. 85	29.5.31	5 p.m.	30.5.31	8 a.m.	—	7.9.31	*
4673	5.6.31	5 p.m.	6.6.31	8 a.m.	—	4.9.31	*
O. 105	6.6.31	5 p.m.	7.6.31	8 a.m.	—	1.9.31	*
188	13.6.31	5 p.m.	14.6.31	8 a.m.	—	31.8.31	*
O. 107	15.6.31	6 p.m.	16.6.31	9 a.m.	—	26.8.31	*
O. 210	18.6.31	4 p.m.	19.6.31	7 a.m.	—	24.8.31	*
E. 85	19.6.31	4 p.m.	20.6.31	7 a.m.	—	24.8.31	*
186	22.6.31	3 p.m.	23.6.31	6 a.m.	—	18.8.31	*

TABLE 8 (EXPERIMENT 2B).

GROUP 7.—EWES SERVED 18 HOURS AFTER THE ONSET OF OESTRUS.

O. 125	19.2.31	4 p.m.	20.2.31	10 a.m.	—	25.3.31	*
O. 165	20.2.31	2 p.m.	21.2.31	8 a.m.	—	30.3.31	*
O. 102	26.2.31	2 p.m.	27.2.31	8 a.m.	—	13.4.31	*
O. 114	1.3.31	2 p.m.	2.3.31	8 a.m.	—	17.4.31	*
O. 101	6.3.31	7 p.m.	7.3.31	1 p.m.	—	28.4.31	*
O. 77	7.3.31	1 p.m.	8.3.31	7 a.m.	—	28.4.31	*
O. 75	9.3.31	7 p.m.	10.3.31	1 p.m.	—	30.4.31	*
G. 115	5.5.31	2 p.m.	6.5.31	8 a.m.	—	27.7.31	*
G. 118	6.5.31	5 p.m.	7.5.31	11 a.m.	—	29.7.31	*
O. 152	7.5.31	6 p.m.	8.5.31	12 noon	—	31.7.31	*

N.B.—See Table 8A Expt. 2B in Appendix.

TABLE 9 (EXPERIMENT 2B).

GROUP 8.—EWES SERVED 21 HOURS AFTER THE ONSET OF OESTRUS.

Ewe No.	Occurrence of Oestrus.		Service 4 Times.		Recur- rence of Oestrus.	Date Killed.	Result of Mating.
	Date.	Time.	Date.	Time.			
O. 153	18.5.31	3 p.m.	19.5.31	12 noon	—	10.8.31	*
189	20.5.31	2 p.m.	21.5.31	11 a.m.	—	17.8.31	*
198	24.5.31	2 p.m.	25.5.31	11 a.m.	—	9.9.31	*
G. 127	25.5.31	10 a.m.	26.5.31	7 a.m.	—	8.9.31	*
O. 182	26.5.31	2 p.m.	27.5.31	11 a.m.	—	7.9.31	*
172	27.5.31	2 p.m.	28.5.31	11 a.m.	—	7.9.31	*
O. 187	29.5.31	12 noon	30.5.31	9 a.m.	—	4.9.31	*
179	29.5.31	5 p.m.	30.5.31	2 p.m.	—	4.9.31	*
O. 189	30.5.31	11 a.m.	31.5.31	8 a.m.	—	4.9.31	*
O. 191	18.6.31	2 p.m.	19.6.31	11 a.m.	10.7.31	—	—

TABLE 10 (EXPERIMENT 2B).

GROUP 9.—EWES SERVED 24 HOURS AFTER THE ONSET OF OESTRUS.

O. 159	18.2.31	10 a.m.	19.2.31	10 a.m.	—	26.3.31	*
O. 136	19.2.31	2 p.m.	20.2.31	2 p.m.	—	27.3.31	*
O. 138	24.2.31	4 p.m.	25.2.31	4 p.m.	—	10.4.31	*
O. 131	4.3.31	10 a.m.	5.3.31	10 a.m.	21.3.31	—	—
O. 108	4.3.31	3 p.m.	5.3.31	3 p.m.	—	24.4.31	*
O. 87	6.3.31	8 a.m.	7.3.31	8 a.m.	—	1.5.31	*
O. 168	2.5.31	1 p.m.	3.5.31	1 p.m.	—	23.7.31	*
O. 174	3.5.31	8 a.m.	4.5.31	8 a.m.	—	24.7.31	*
O. 43	7.5.31	3 p.m.	8.5.31	3 p.m.	—	4.8.31	*
O. 160	8.5.31	6 p.m.	9.5.31	6 p.m.	—	6.8.31	*

TABLE 11 (EXPERIMENT 2B).

GROUP 10.—EWES SERVED 27 HOURS AFTER THE ONSET OF OESTRUS.

O. 131	8.5.31	12 noon	9.5.31	3 p.m.	12.7.31	—	—
O. 104	19.5.31	11 a.m.	20.5.31	2 p.m.	—	13.8.31	*
O. 183	24.5.31	2 p.m.	25.5.31	5 p.m.	—	8.9.31	*
O. 184	25.5.31	7 a.m.	26.5.31	10 a.m.	—	8.9.31	*
O. 158	28.5.31	1 p.m.	29.5.31	4 p.m.	—	7.9.31	*
2396	10.6.31	10 a.m.	11.6.31	1 p.m.	15.7.31	—	—
O. 186	15.6.31	2 p.m.	16.6.31	5 p.m.	—	26.8.31	*
MBA 33	19.6.31	10 a.m.	20.6.31	1 p.m.	—	24.8.31	*
MBA 42	21.6.31	10 a.m.	22.6.31	1 p.m.	—	24.8.31	*
O. 81	25.5.31	10 a.m.	26.5.31	1 p.m.	Stolen, no	result.	—

TABLE 12 (EXPERIMENT 2B).

GROUP 11.—EWES SERVED 30 HOURS AFTER THE ONSET OF OESTRUS.

O. 89	18.2.31	10 a.m.	19.2.31	4 p.m.	—	30.3.31	*
O. 146	19.2.31	11 a.m.	20.2.31	5 p.m.	—	27.3.31	*
O. 70	21.2.31	10 a.m.	22.2.31	4 p.m.	—	31.3.31	*
O. 64	24.2.31	4 p.m.	25.2.31	10 a.m.	—	9.4.31	*
O. 140	25.2.31	11 a.m.	26.2.31	5 p.m.	—	14.4.31	*
O. 94	26.2.31	1 p.m.	27.2.31	7 p.m.	17.3.31	—	—
O. 120	9.3.31	9 a.m.	10.3.31	3 p.m.	—	30.4.31	*
O. 166	5.5.31	8 a.m.	6.5.31	2 p.m.	—	28.7.31	*
O. 179	6.5.31	10 a.m.	7.5.31	4 p.m.	—	31.7.31	*
2397	8.5.31	7 a.m.	9.5.31	1 p.m.	—	4.8.31	*

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TABLE 13 (EXPERIMENT 2B).

GROUP 12.—EWES SERVED 33 HOURS AFTER THE ONSET OF OESTRUS.

Ewe No.	Occurrence of Oestrus.		Service 4 Times.		Recur- rence of Oestrus.	Date Killed.	Result of Mating.
	Date.	Time.	Date.	Time.			
G. 174	24.5.31	7 a.m.	25.5.31	4 p.m.	14.7.31	—	*
G. 178	25.5.31	7 a.m.	26.5.31	4 p.m.	—	7.9.31	—
2398	26.5.31	7 a.m.	27.5.31	4 p.m.	—	7.9.31	*
MX 11	7.6.31	11 a.m.	8.6.31	8 p.m.	—	1.9.31	*
183	8.6.31	10 a.m.	9.6.31	7 p.m.	—	31.8.31	*
O. 84	9.6.31	11 a.m.	10.6.31	8 p.m.	—	31.8.31	*
O. 90	14.6.31	8 a.m.	15.6.31	5 p.m.	—	27.8.31	*
O. 197	15.6.31	10 a.m.	16.6.31	7 p.m.	—	26.8.31	*
181	16.6.31	7 a.m.	17.6.31	4 p.m.	4.7.31	—	—
180	19.6.31	10 a.m.	20.6.31	7 p.m.	—	24.8.31	*

TABLE 14 (EXPERIMENT 2B).

GROUP 13.—EWES SERVED 36 HOURS AFTER THE ONSET OF OESTRUS.

O. 107	17.2.31	7 p.m.	19.2.31	7 a.m.	6.3.31	—	—
O. 164	23.2.31	7 a.m.	24.2.31	7 p.m.	12.3.31	—	—
O. 66	25.2.31	7 a.m.	26.2.31	7 p.m.	—	1.4.31	*
O. 119	25.2.31	6 p.m.	27.2.31	6 a.m.	—	8.4.31	*
O. 63	2.3.31	9 a.m.	3.3.31	9 p.m.	20.3.31	—	—
O. 149	5.3.31	7 p.m.	7.3.31	7 a.m.	—	29.4.31	*
O. 171	3.5.31	7 a.m.	4.5.31	7 p.m.	—	24.7.31	*
G. 122	6.5.31	7 a.m.	7.5.31	7 p.m.	—	31.7.31	—
O. 169	6.5.31	6 p.m.	8.5.31	8 a.m.	11.7.31	—	—
G. 107	7.5.31	6 p.m.	9.5.31	6 a.m.	—	4.8.31	*

TABLE 15 (EXPERIMENT 2B).

GROUP 14.—EWES SERVED 39 HOURS AFTER THE ONSET OF OESTRUS.

G. 179	8.5.31	5 p.m.	10.5.31	8 a.m.	12.7.31	—	—
2391	8.5.31	6 p.m.	10.5.31	9 a.m.	—	17.7.31	—
G. 132	23.5.31	3 p.m.	25.5.31	6 a.m.	—	9.9.31	—
MX 26	6.6.31	5 p.m.	8.6.31	8 a.m.	—	1.9.31	*
G. 176	11.6.31	4 p.m.	13.6.31	7 a.m.	17.7.31	—	—
O. 95	15.6.31	4 p.m.	17.6.31	7 a.m.	—	25.8.31	*
4669	16.6.31	4 p.m.	18.6.31	7 a.m.	—	24.8.31	*
182	19.6.31	3 p.m.	21.6.31	6 a.m.	8.7.31	—	—
E. 88	21.6.31	2 p.m.	23.6.31	5 a.m.	—	18.8.31	*
O. 211	21.6.31	11 p.m.	23.6.31	2 p.m.	—	18.8.31	*

TABLE 16 (EXPERIMENT 2B).

GROUP 15.—EWES SERVED 42 HOURS AFTER THE ONSET OF OESTRUS.

O. 68	23.2.31	1 p.m.	25.2.31	7 a.m.	—	1.4.31	*
O. 118	24.2.31	4 p.m.	26.2.31	10 a.m.	14.3.31	—	—
O. 157	25.2.31	5 p.m.	27.2.31	11 a.m.	14.3.31	—	—
O. 162	3.3.31	1 p.m.	5.3.31	7 a.m.	—	27.4.31	*
O. 176	3.5.31	2 p.m.	5.5.31	8 a.m.	—	27.7.31	*
O. 73	3.5.31	5 p.m.	5.5.31	11 a.m.	—	27.7.31	—
O. 175	4.5.31	5 p.m.	6.5.31	11 a.m.	—	27.7.31	*
M. 926	5.5.31	2 p.m.	7.5.31	8 a.m.	—	28.7.31	—
G. 177	6.5.31	3 p.m.	8.5.31	9 a.m.	—	31.7.31	*
G. 175	7.5.31	1 p.m.	9.5.31	7 a.m.	11.7.31	—	—

* In all tables * signifies fertilized; — not fertilized.

N.B.—See Table 16A Expt. 2B in Appendix for ewes served 45 hours after the onset of oestrus.

The following table has been compiled from the data given in tables 2 to 16:—

TABLE 17 (EXPERIMENT 2B).

Group No.	Time of Services.	Number showing Recurrence of Oestrus.	Number non-pregnant on Slaughtering.	Total not Fertilized.	Per cent. Fertilized.
	Hours.				
1.....	0	1	0	1	90
2.....	3	3	2	5	50*
3.....	6	2	0	2	80
4.....	9	0	0	0	100
5.....	12	1	1	2	80
6.....	15	0	0	0	100
7.....	18	0	0	0	100*
8.....	21	1	0	1	90
9.....	24	1	0	1	90
10.....	27	2	0	2	77.8
11.....	30	1	0	1	90
12.....	33	2	1	3	70
13.....	36	4	1	5	50
14.....	39	3	2	5	50
15.....	42	3	2	5	50
TOTALS..		24	9	33	

* See Appendix, Tables 3A and 8A.

With regard to the above table, it will be observed that the percentage of ewes fertilized decreased very decidedly beyond the 30th hour after the onset of oestrus. In this connection it must be recorded that a number of ewes in groups 12-15 either definitely refused the ram and had to be forced to service, or showed strong indications of oestrus passing over. In group 12, 3 ewes had to be held; in group 13, 3; group 14, 6; in group 15, 6. Seven out of these 18 ewes which were forced to service were fertilized. Quinlan and Maré (1931) showed that oestrus in Merino sheep lasts on an average from 36-48 hours. This finding is confirmed by the above facts, viz., that a number of ewes had to be forced to service from the 33rd hour after the onset of oestrus.

The following table shows the results obtained with ewes mated in two different groups:—

TABLE 18 (EXPERIMENT 2B).

Ewe No.	First Service At.	Result.	Second Service At.	Result.
	Hours.		Hours.	
O. 158.....	0	Not fertilized	27	Fertilized.
O. 147.....	6	"	12	"
O. 105.....	6	"	15	"
O. 106.....	12	"	3	Not fertilized.
O. 131.....	24	"	27	"
O. 94.....	30	"	9	Fertilized.
O. 107.....	36	"	15	"
O. 164.....	36	"	9	"
O. 63.....	36	"	6	"
O. 118.....	42	"	3	"
O. 157.....	42	"	0	"

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The following table shows the intensity of services by the rams on dates involving all unsuccessful services. A series of other dates are included in the table for comparison:—

TABLE 19 (EXPERIMENT 2B).

Date.	No. of Services by Rams.			No. of Ewes Served.	No. of Ewes not Fertilized.	Order of Service.	Class.
	T. 413.	T. 417.	W. 31.				
							Hours.
18.2.31	6	6	0	3	0	—	—
19.2.31	8	8	0	4	1	1st	36
20.2.31	8	8	0	4	0	—	—
23.2.31	8	8	0	4	1	2nd	12
24.2.31	3	3	0	2	1	2nd	36
26.2.31	8	8	0	4	1	2nd	42
27.2.31	8	8	0	4	2	4th and 2nd	30 and 42
28.2.31	2	2	0	1	1	1st	6
2.3.31	8	8	0	4	0	—	—
3.3.31	6	6	0	3	3	1st, 2nd, 3rd	0, 6, and 36
4.3.31	4	4	0	2	0	—	—
5.3.31	8	8	0	4	1	2nd	24
5.5.31	4	2	2	2	1	2nd	42
6.5.31	6	4	6	4	1	4th	12
7.5.31	7	5	8	5	1	1st	42
8.5.31	7	6	7	5	2	1st and 2nd	36 and 42
9.5.31	6	7	7	5	2	4th and 2nd	27 and 42
10.5.31	4	4	4	3	2	1st and 2nd	29 and 39
18.5.31	0	2	2	1	1	1st	3
25.5.31	5	5	6	4	2	3rd and 1st	33 and 39
26.5.31	6	5	5	4	1	4th	33
30.5.31	6	6	8	5	1	3rd	3
3.6.31	0	0	0	0	0	—	—
4.6.31	1	1	2	1	1	1st	3
5.6.31	0	0	0	0	0	—	—
11.6.31	5	6	5	4	1	2nd	27
13.6.31	1	2	1	1	1	1st	39
16.6.31	4	4	4	3	0	—	—
17.6.31	4	3	5	3	1	2nd	33
18.6.31	4	4	4	3	1	2nd	3
19.6.31	5	5	6	4	2	3rd and 2nd	3 and 21
20.6.31	3	4	5	3	0	—	—
21.6.31	2	2	0	1	1	1st	39

NOTE.—A study of the above table will indicate that failure to impregnate cannot be ascribed to over-taxation of rams. Particular attention may be drawn to the 4th of June, when two rams served only one ewe and failed to fertilize her, in spite of their having had a complete rest on the previous day.

The following table shows the distribution of services during the specified periods of the day:—

TABLE 20 (EXPERIMENT 2F).

Period.	Number of Ewes Served.	Number Fertilized.	Per cent. Fertilized.
6 a.m.—10 a.m.....	52	40	77·0
11 a.m.—3 p.m.....	43*	35	83·3
4 p.m.—9 p.m.....	55	41	74·5

* One ewe was stolen in this group.

NOTE.—As the work was carried out in the open, and partly during the hot summer months with temperatures up to 100° F. in the shade, the possibility existed that the rams might have been more sluggish during the heat of the day and consequently failed to copulate with sufficient vigour. The results as shown in Table 20, however, indicate that the rams could not have been affected adversely by the heat. From 11 a.m.—3 p.m., which is always the hottest part of the day, the rams actually fertilized a higher percentage of ewes than during the other periods.

The following table indicates the number of occurrences of oestrus which commenced during the periods indicated:—

TABLE 21 (EXPERIMENT 2B).

6 a.m. to 12 noon.	1 p.m. to 6 p.m.	6 p.m. to 6 a.m.
75	85	95

NOTE.—It is computed that in 62·7 per cent. of cases oestrus commenced during the day. These figures studied in conjunction with Table 20 (Experiment 2A) indicate that whereas at Ermelo approximately 40 per cent. of cases of oestrus occurred during the day time, at Grootfontein the reverse held good. One may, therefore, justly conclude that the occurrence of oestrus in a flock of sheep is uniformly distributed over the 24 hours.

SUMMARY OF EXPERIMENT 2 (A AND B).

The following table has been compiled from data given in table 15 (Experiment 2A) and table 17 (Experiment 2B):—

TABLE 22 (EXPERIMENTS 2A and 2B).

Group No.	Time of Service.	Number Served.	Percentage Fertility.		
			At Grootfontein.	At Ermelo.	Combined.
	Hours.				
1.....	0	20	90	70	80
2.....	3	20	50	90	70*
3.....	6	20	80	100	90
4.....	9	20	100	80	90
5.....	12	20	80	70	75
6.....	15	20	100	90	95
7.....	18	20	100	70	85*
8.....	21	20	90	90	90
9.....	24	20	90	80	85
10.....	27	20	77·8	80	79
11.....	30	20	90	90	90
12.....	33	20	70	60	65
13.....	36	20	50	50	50
14.....	39	10	50	—	50
15.....	42	10	50	—	50
	45*				

NOTE.—A study of the above table reveals marked variations at the two centres of investigation in respect of the fertility of the ewes in corresponding groups. It is doubtful, however, whether these variations are of significance on account of the small number of individuals in each group. The groups where great variation occurs are being repeated.

* See Appendix.

DISCUSSION.

Hammond and Asdell (1926) point out that the deteriorating influence of a prolonged stay in the genital tract may be sufficient to kill off sperms of low vitality or at least destroy their fertilizing power in a relatively shorter period than vigorous sperms. Crew (1922) and Moore (1924) suggest that this deteriorating influence may be the higher temperature experienced in the female genitalia. This

appears highly probable in view of the work of Crew (1926). He has shown that the scrotum is a temperature-regulating mechanism necessary for the efficient elaboration of functional spermatozoa. It is, therefore, probable that the environmental change from the testicle to the higher temperature of the female genitalia may be a cause of relatively rapid death of sperms as compared with their normal environmental habitat in the male.

There appears to be some other factor, however, besides actual elevation of temperature in their new environment, which is detrimental to the life of the sperm. In experiments now in progress it appears to be definitely proved that sperm life is longer in the ostium uterinum of the ewe than in the other divisions of the genitalia.

Hammond and Asdell (1926) suggest the presence of leucocytes as another possible explanation. They state: "Another possible cause of the short vitality of the sperm in the female tract is the presence of leucocytes in these organs. In semen obtained from does a few days after parturition, one of the writers has, in conjunction with Mr. A. Walton, observed the sperms clustering around leucocytes and apparently attempting to fertilize them; in this way the majority of the sperms would become flocculated after remaining for some time in the female tract. There is, as is well known, difficulty in breeding from females which have excessive discharge of leucocytes from the tract."

Our observations on the sperms which have remained some hours in the genitalia of the ewe confirm this observation. This occurs in all the departments of the genitalia. Sperm cells can be seen penetrating leucocytes and epithelial cells in an apparent attempt to fertilize them or in a process of phagocytosis by the leucocytes.

Hammond and Asdell (1926) maintain that in spite of large numbers of spermatozoa in the semen of normal male rabbits it is quite possible that small sized litters may be produced by males whose sperms are, for some reason, deficient in vigour, although still numerous and motile. There appears little doubt that this suggestion is also possible in other animals; in other words there may be no correlation between promptness and vigour of service, or the seminal picture, with the percentage of resulting pregnancies [Donham, Simms, and Shaw (1931)]. Our experiences with Merino rams have definitely convinced us that the ram is no exception to this rule [Quinlan and Maré (1931)]. It appears, from observations made on 13 rams, used for mating by the controlled service method, that certain individuals emit sperms with lower fertilizing power than others. Some of the rams used were weak servers. This applies to rams which are advancing in years, after sickness, or on account of obesity, when the vitality is lowered, but it is also seen in rams which appear in good hard condition in which there is no apparent clinical cause.

In this connection, if Table 2 be consulted, it will be observed what a difference in fertilizing power may exist between clinically normal rams used on clinically normal ewes when the controlled service method was carried out under identical conditions.

TABLE 2.

RAM No.	First Service.				Second Service.				Third Service.				Fourth Service.				All Services.			
	No. Served.	No. Fertilized.	No. not Fertilized.	Percentage Fertilized.	No. Served.	No. Fertilized.	No. not Fertilized.	Percentage Fertilized.	No. Served.	No. Fertilized.	No. not Fertilized.	Percentage Fertilized.	No. Served.	No. Fertilized.	No. not Fertilized.	No. of Individuals Served.	No. Fertilized.	No. not Fertilized.	Percentage Fertilized.	
1....	26	22	4	84.6	2	2	0	100	—	—	—	—	—	—	—	26	24	2	92.3	
2....	23	19	4	82.6	2	1	1	50	—	—	—	—	—	—	—	23	20	3	87.0	
3....	16	13	3	81.2	—	—	—	—	—	—	—	—	—	—	—	16	13	3	81.2	
4....	25	19	6	76.0	6	5	1	83.3	1	1	0	100	—	—	—	25	23	0	100.0	
5....	96	68	28	70.8	13	6	7	46.2	1	1	0	100	—	—	—	96	75	21	78.1	
6....	65	39	26	60.0	18	6	12	33.3	5	1	4	20	0	1	0	65	46	19	70.8	
7....	33	19	14	57.6	10	6	4	60.0	1	0	1	0	0	—	—	33	25	8	75.8	
8....	25	14	11	56.0	5	3	2	60.0	—	—	—	—	—	—	—	25	17	8	68.0	
9....	55	30	25	54.6	17	9	8	52.9	1	1	0	100	—	—	—	55	40	15	72.8	
10....	40	13	27	32.5	26	17	9	65.3	1	1	0	100	—	—	—	40	31	9	77.5	
11....	21	2	19	9.5	6	3	3	50.0	—	—	—	—	—	—	—	21	5	16	23.8	
12....	54	4	50	7.4	37	8	29	21.6	4	3	1	75.0	—	—	—	54	15	39	27.8	
13....	46	0	46	0	45	4	41	8.8	32	8	24	25.0	4	0	4	46	12	34	26.1	
TOTAL	525	262	263	49.92	187	70	117	37.44	46	16	30	34.8	5	0	5	525	348	177		

No account is taken here of the behaviour of the ewe, i.e. a ewe may figure under First Service to Ram No. 5, but she may also figure under Ram No. 6 as having been served by him once or even twice but not fertilized at those services.

It may be indicated that this low percentage fertilizing power in rams may be quite temporary. It may differ during successive seasons and even during the same season. In other words, it appears that sperms of low fertilizing power may be emitted by rams which appear normal, but for some reason, either local or general, produce weakened sperms. An experiment, now in progress, on the artificial insemination of ewes with sperms kept for intervals after ejaculation should give some definite information on this subject.

It, therefore, becomes apparent what importance the knowledge of the time of ovulation in sheep has to the vitality of spermatozoa in the genitalia, if the controlled service method of mating is to be universally adopted, and the highest percentage of fertility is to be obtained when one service only is allowed at a definite hour during oestrus.

Marshall and Hammond (1926) state that the length of time the sperms of the rabbit can live varies in different males. Lewis (1911) working with boars indicates that there is great variation in the vitality of the sperm cells from the same individual at different times. It is quite possible that a similar variation of viability may occur in rams. This possibility, coupled with incomplete coitus and delay in the genital tract awaiting the arrival of an ovum, are factors which cannot be lost sight of in connection with fertility in sheep [Quinlan and Maré (1931)]. Another factor which bears the closest relation to the correlation between the time of copulation and ovulation in fertility is the possibility of the detrimental influence of genital secretions on the spermatozoa. It has already been pointed out that a variation in the duration of fertilizing power may exist in the sperms of different males. Coupled with this there may be an environmental factor in the genitalia of different females which acts detrimentally on the sperms introduced, such as harmful secretions, and high temperature, especially experienced in this country, where temperatures of 105° F. and even higher are not uncommonly registered in Merino sheep in the afternoon during the summer months [Quinlan and Maré (1932)].

Löw (1902) has observed that the vaginal secretion of the rat is harmful to the motility and vitality of spermatozoa. On the other hand the uterus secretion is favourable. The vaginal mucosa is anti-chemiotactic, while the uterine mucosa is chemiotactic. Long and Evans (1922) have also indicated the detrimental influence of contact with uterine secretion to spermatozoa in the rat. Kugota (1929), working on the mouse, states: "Der Einfluss des Uterus-Saftes auf die Lebensdauer der Spermatozoen ist in den Perioden des oestrischen Zyklus verschieden. In der zweiten Periode wirkt er höchst günstig. Diese Wirkung beginnt schon in der ersten Periode und ist in der dritten Periode plötzlich sehr gering. Diese Einwirkung auf die Lebensdauer der Spermatozoen scheint um so günstiger zu sein, je stärker der Uterus-Saft konzentriert ist. In der vierten Periode und im Dioestrus können wir weder eine günstiger noch eine nachteilige Wirkung finden." "Die Lebensdauer der Spermatozoen ist bei grösserem (schwererem) Uterus grösser als bei kleinem (leichterem)."

Wester (1921) states that the spermatozoa of the bull or goat-ram taken from the female vagina immediately after copulation may remain motile *in vitro* for more than 24 hours, and even after several days when kept at room temperature, while sperms which were allowed to remain a short time in contact with the vaginal secretion were almost all dead in 5 or 6 hours. Renkert (1913) also working with cattle, arrived at the same conclusions as Wester, while Hutschenreiter (1915) working with mares also had similar results.

It will be evident from those workers' observations that a study of the vitality of spermatozoa *in vitro* is of little practical value and reliable data can be obtained only by a study of sperms in their natural environmental habitat after ejaculation from the male. Further, since motile sperms do not always retain their fertilizing capacity, motility does not necessarily imply fecundity, so that the practical aspect is of the utmost importance in breeding, that is the time relation between coitus and ovulation when one service only is allowed. Details concerning this aspect of breeding have been shown in data published in Experiment 2. Referring to the time after ovulation during which the ovum remains capable of fertilization in the rabbit, Hammond and Marshall (1925), state that it is usually confined to 2 to 4 hours after it leaves the follicle; at the most 6 hours after ovulation. [Hammond's (1932) work on the mare would also indicate the short duration of the fertilizable availability of the ovum after ovulation.] Pincus (1930) has confirmed these observations by killing female rabbits, which had been mated to sterile bucks, to produce ovulation and then to fertile bucks 14 hours later, and submitting the ova to histological examination. All ova from such a mating proved to be non-fertilized. He also found that the ova from a rabbit killed 17 hours 40 minutes after a sterile mating, followed 14 hours later by a fertile mating were not fertilized. In another rabbit in which a sterile mating was followed 12 hours later by a fertile mating he found a single sperm head in the zona pellucida of one of the two ova recovered. Pincus in discussing the reasons for the short duration during which the ova are available for fertilization says: "It is obvious that more information than is at present available is needed for ascertaining just why the ova remain fertilizable for such a short time. An exhaustive investigation would certainly be worth while in view of the pertinence of the ensuing results to the general problem of the fertilizing capacity of the ovum."

He indicates that the possible causes may be some process going on in the tubes, which is responsible for non-entry of the sperms, or that the sperms have not reached the ovum. In considering the first possibility he suggests that the ova which have separated out from the granulosa mass pass so rapidly through the main body of sperm that it is impossible for sufficient sperms to attach themselves to these ova to make the chance of penetration likely, or perhaps when the sperms reach the ova they are already made impenetrable by the surrounding capsule of albumen which is described.

Lewis (1911) attempted to establish the duration of the fertilizable vitality of the ovum in the sow by breeding at different lengths of time after the period of heat ended. Two out of seven services on

the day after the heat period had passed were successful. Twenty-seven other services given at different intervals following the disappearance of oestrus were unsuccessful. From his observations he concludes that in most cases the ovum appears to lose its power of being fertilized within 48 hours after liberation from the ovary.

In a few cases the authors have been successful in the case of sheep with which forced service had to be used. These services, however, were performed a short time after the ewe no longer stood for the ram.

It is evident, from the results which have been obtained that the ovum of the ewe does not remain long available for fertilization. If Table 1 (Experiment 1) be consulted, it will be observed that motile sperms have been observed in the cervix and the uterus of a ewe up to forty-eight hours following coitus. These sperms, however, were only very sluggishly motile and represented only 8 per cent. of the sperms seen in the cervical smears; 92 per cent. were non-motile; of 4 sperms seen in the smears from the uterine horns near the apex at the 48th hour after coitus, 3 were sluggishly motile. This is the longest period after coitus at which living sperms have been seen. It is highly improbable that these sluggishly motile sperms are capable of impregnating an available ovum, but the work now being continued should clear up this question. Table 1 (Experiment 1) indicates the variation which may exist in the duration of vitality in the sperms of the same rams in individual ewes. It appears that the sperms are highly sensitive to the secretions in the genitalia. They show motility for a short period only in the vagina. Those which remain in the vagina begin to lose their motility after a period of 3 to 6 hours. There is a gradual loss of vitality until there are no more motile sperms at the 18th to the 24th hour. Occasionally after this period there are isolated cases in which very sluggishly motile sperms are seen.

The sperms are not deposited in the cervix during copulation, but they may be found there within a period of 15 minutes following coitus. Very large numbers of sperms enter the cervix and here they remain motile longer than in the other divisions of the genitalia. Fairly actively motile sperms have been seen here 42-45 hours after coitus, and sluggishly motile sperms have been encountered after 48 hours. It has been pointed out that much variation exists in the duration of motility in individuals, but 99.5 per cent. of motile sperms have been seen in one case in the cervix after a stay of 45 hours. In this case the sperms were numerous in the cervix, but no doubt the larger percentage had already undergone spermalysis.

It appears that of all the compartments of the genitalia the secretion of the cervix is most favourable for the vitality of the spermatozoa in the Merino sheep. It is possible that the cervical canal acts as a reservoir for spermatozoa after copulation, in which the spermatozoa stored during the earlier part of oestrus are kept alive, and pass upward from here in small numbers in search of an available ovum until the end point of their vitality is reached.

Our observations indicate that only relatively small numbers of sperms ever reach the pars indivisa of the uterus. Still fewer reach the tubes. In fact in the latter division of the genitalia it is often necessary to examine a large number of microscopic fields before finding a sperm. As a rule, however, they are easily found in the uterine extremity of the tube in fresh preparations.

Spermatozoa which have lost their power of movement rapidly begin to show morphological changes. There appears to be a spermatolytic process so that inside a few hours dead spermatozoa are no longer intact. They become disintegrated and rapidly disappear.

Quinlan and Maré (1931) pointed out that the time of ovulation in the Merino sheep in South Africa is between the 36th and 40th hour following the commencement of oestrus. If Table 22 (Experiments 2A and 2B) be consulted it will be seen that of 20 sheep served immediately at the onset of oestrus 80 per cent. proved to be pregnant. Assuming that the ovum is available for fertilization between the 36th to the 40th hour, plus a period of a few hours taken to enter the Fallopian tube, spermatozoa are definitely capable of fertilization for 36 to 42 hours after being deposited in the vagina. However, as a rule, there is a higher percentage fertility if copulation is allowed between the 6th and the 30th hour after the onset of oestrus.

Table 22 (Experiments 2A and 2B) indicates that coitus following the 30th hour of oestrus shows a decreasing fertility as the interval since its onset becomes longer. These experiments have shown that the sperm cell can reach the Fallopian tube within six hours after being deposited in the vagina. It is, therefore, apparent that sperms deposited at the 33rd hour following the onset of oestrus would be available to fertilize an ovum at the 39th hour; sperms deposited at the 36th hour would be available at the 42nd hour, but fertilization drops as low as 60 per cent. at the 33rd hour, and as low as 50 per cent. at the 36th hour following the onset of oestrus. This indicates that the ovum rapidly loses its vitality and is available for fertilization for a few hours only after ovulation.

Work on the vitality of spermatozoa in the different divisions of the female genitalia is in progress, but it has not yet reached a stage fit for publication. Sperms have been transferred from the vagina after copulation to the uterine horns and tubes after exposure, through a laparotomy opening. These experiments support the observation that the sperms survive much longer in the cervix than in the other divisions of the female genitalia.

CONCLUSIONS.

(1) The ejaculated semen of the ram is deposited in the cranial extremity of the vagina and quickly reaches the first few mm. of the cervical canal. It is not deposited in the ostium uterinum or in the pars indivisa of the uterus.

(2) Spermatozoa may be found in the pars indivisa of the uterus as early as 15 minutes after coitus.

(3) Relatively few of the spermatozoa, which enter the cervical canal from the vagina reach the uterus, and only a small percentage of those which enter the uterus reaches the Fallopian tubes.

(4) Spermatozoa may reach the abdominal extremity of the Fallopian tubes within 6 hours following coitus.

(5) The majority of spermatozoa found in the vagina are non-motile, following a stay of 12 hours, although a few living but sluggishly motile sperms may still be present in some cases after 24 hours.

(6) Live sperms may be found in the cervical canal up to the 48th hour after coitus.

(7) Living sperms seen in the uterus, uterine horns, and the Fallopian tubes, at long intervals after copulation, do not reach these divisions of the genitalia shortly after mating and remain alive there. They result from a continuous issue from the cervix, pending the arrival of an available ovum, during the oestrus period. (The longest interval after which living sperms have been found in the isolated tubes after artificial transmission from the vagina has been 9 hours.)

(8) The motility of spermatozoa becomes reduced after a stay of 12 hours in the genital tract of the ewe. It gradually becomes decreased until after a stay just over 48 hours no living sperms have been found.

(9) After a time, some sperms begin to show sluggish movement and gradually cease to move. They then begin to show morphological changes and undergo spermalysis.

(10) After a prolonged stay in the genital tract the sperms may be seen clustering around leucocytes and epithelial cells as if attempting to impregnate them.

(11) It is apparent that the spermatozoa of healthy virile rams are capable of satisfactorily impregnating an available ovum from the onset of oestrus until 30 hours afterwards; within this period fertility ranges between 70 and 100 per cent.

(12) Lowered fertility follows services after the 30th hour following the onset of oestrus. This must be interpreted as due to the short duration of fertilizable vitality of the liberated ovum rather than to the lack of vitality of the spermatozoon, as indicated by the fact that 80 per cent. of sheep served at the onset of oestrus were fertilized.

(13) The cervical canal would appear to act as a reservoir for spermatozoa awaiting the availability of an ovum. Its anatomical structure would support this view. There is no doubt that the cervical secretion is highly favourable to the vitality of spermatozoa in the genital tract of the ewe, when compared with vaginal, uterine and tubal secretions.

(14) Fertility in the Merino sheep in South Africa is comparatively low, probably not exceeding 90 per cent.

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LITERATURE.

- ANDERSON, W. S. (1922). The Vitality of spermatozoa in some domestic animals. *Bull.* 239, *Kentucky Agric. Expt. Stn. Univ. Kentucky*.
- BAILEY, F. R., AND MILLER, A. M. (1911). *Textbook of Embryology*: William Wood & Co., New York.
- COURRIER, R. (1922). Le cycle genital de la femelle chez certains mammiferes hibernants. *C.R. Soc. Biol.* 87.
- COURRIER, R. (1923). Cycle animal de la glande interstitielle du testicule des les cheiroptires. *C.R. Soc. Biol.* 88.
- CREW, F. A. E. (1926). The Scrotum. A temperature regulating mechanism. *Verhandl. I. Inter. Kongr. f. Sexualforsch.* Oktober 1926. Verlag, Markus u. Weber, Berlin und Köln, 1927.
- DONHAM, C. R., SIMMS, B. T., AND SHAW, J. N. (1921). Fertility studies in the Bull. *Jl. Amer. Vet. Med. Assn.* 31, 5.
- GILES, A. E. (1919). Sterility in Women. Hodder & Stoughton, London.
- HAMMOND, J. (1928). Die Kontrolle der Fruchbarkeit bei Tieren. *Züchtungskunde* 3, 11.
- HAMMOND, J. (1930). The effect of temperature on the survival in vitro of rabbit spermatozoa obtained from the vagina. *Br. Jl. Expt. Biol.*, Vol. 7, No. 2.
- HAMMOND, J. (1931). Personal communication.
- HAMMOND, J., AND ASDELL, A. S. (1926). The vitality of the spermatozoa in the male and female reproductive tract. *Br. Jl. Expt. Biol.* 4, 2.
- HAMMOND, J., AND MARSHALL, F. H. A. (1925). Reproduction in the Rabbit. Edinburgh.
- HAUSSMANN, D. (1879). Ueber das Verhalten der Samenfäden in den Geschlechtsorganen des Weibes. Berlin. Cit. Giles.
- HIRSCHFIELD. Cit. Bab. (1908) Konzeption, Menstruation und Schwangerschaft, Leipzig & Berlin.
- HOEHNE AND BEHNE (1913). *Verhandl. Deutsch. Gesell. f. Gynaek.* cit. Hammond & Asdell.
- HOEHNE & BEHNE (1914). *Zentralblatt. f. Gynaek.* 38, 1. cit. Hammond & Asdell.
- HÜHNER, M. (1913). Sterility in the Male and Female. New York. Cit. Giles.
- HUTSCHENREITER, K. (1915). Vagina und Uterus der Pferdestute in ihren Reaktionen auf den Koitus. *Wiener Tierärztl. Monatsschr.* 2.
- KNAUS, H. (1913). Über den Zeitpunkt der Konzeptionsfähigkeit des Weibes. *M. Med. W'och.* 78, 9.
- KUGOTA, T. (1929). Der Einfluss des Uterussaftes in verschiedenen Perioden des oestrischen Zyklus auf die Lebensdauer der Spermatozoen. *Zeitsch. Zell. u. Mikros. Anat.* 9, 3.
- LONG, A., AND EVANS, M. (1922). The Oestrous Cycle in the Rat and its Associated Phenomena. *Mém. Univ. Calif.*
- LEWIS (1911). The Vitality of the reproductive cells. *Bull.* 96 *Agric. Expt. Stn. Oklahoma Agric. College*.
- LÖW, O. (1902). Die Chemotaxis der Spermatozoen im weiblichen Genitaltrakt. *Sitzgsber. Akad. Wiss. Wien Math.-Naturwiss. Kl.* 3, No. 3.

- MARSHALL, F. H. H., AND HAMMOND, J. (1926). The Physiology of Animal Breeding, with special reference to the Problem of Fertility. *Min. Agric. Fish. Research Monograph*, No. 2.
- McKENZIE, F. F., AND PHILLIPS, R. W. (1931). Some Observations on the Oestral Cycle in the Sheep. *Record of Proceedings, Annual Meeting American Society of Animal Production, Univ. Missouri and U.S.A. Dept. Agric.*
- MOORE, C. R. (1924). Properties of the Gonads as Controllers of Somatic and Psychical Characteristics. Cit. Hammond & Asdell. *Amer. Jl. Anat.* 34, pp. 269-337.
- MOORE, C. R. (1924). *Endocrinology* 8, p. 493. Cit. Hammond & Asdell.
- PINCUS, P. (1930). Observations on the Living Eggs of the Rabbit. *Proc. Roy. Soc. Biol.* 107, p. 749.
- QUINLAN, J., AND MARE, G. S. (1931). The Physiological changes in the Ovary of the Merino Sheep in South Africa, and their Practical Application in Breeding. *17th Rep. D.V.S. and A.I., U. of S. Africa.*
- QUINLAN, J., AND MARE, G. S. (1932). The normal temperature of Merino Sheep in the Karroo. *18th Rept. D.V.S. and A.I., U. of South Africa.*
- RENKERT (1913). Diss. Stuttgart, cit. Wester.
- REDENZ, E. (1929). Das Verhalten der Säugetierspermatozoen zwischen Begattung und Befruchtung. *Z. Zellforsch.* 9, 4.
- SABOTTA, J. (1895). Die Befruchtung und Züchtung des Eies der Maus. *Arch. Mikros. Anat.* 45, 15.
- SIEGEL, P. W. (1916). Bedeutung des Kohabitationstermines für die Befruchtungsfähigkeit der Frau, und für die Geschlechtsbildung des Kindes. *Münch. Med. Woch.* 63.
- WALTON, A. (1926). Preservation of Mammalian Spermatozoa *Nature*, No. 118, p. 265.
- WALTON, A. (1927). The Relation between "Density" of Sperm-suspension and Fertility as determined by Artificial Insemination of Rabbits. *Proc. Rl. Socy.* B. 101.
- WALTON, A. (1930). The Effect of Temperature on the Survival in vitro of Rabbit Spermatozoa obtained from the Vas Deferens. *Br. Jl. Expl. Biol.* Vol. 7, No. 2.
- WALTON, A., HAMMOND, J., AND ASDELL, A. S. (1928). On the vitality of spermatozoon in the male and female genital tracts and outside the body. *Verhandl. I. Inter. Kongr. f. Sexualforsch.* 2, 5. Verlag. Markus und Weber, Berlin und Köln. *Ztschr. Züchtung.* Vol. 14, No. 2, 1929.
- WESTER, J. (1921). Eierstock und Ei. Befruchtung und Unfruchtbarkeit bei den Haustieren. Berlin.
- WOLF, C. G. L. (1921). The Survival of Motility in Mammalian Spermatozoa. *Jl. Agric. Sc.* No. 11, p. 310.
- YOICHEM, D. E. (1929). Spermatozoon life in the Female Reproductive Tract of the Guinea-pig and Rat. *Biol. Bull. Mar. Biol. Lab., Woods-Hole,* Vol. 56, 4.
- YOICHEM, D. E. (1930). A study of the Motility and Resistance of Rat Spermatozoa at different levels in the Reproductive Tract. *Anat. Record.* No. 45.

APPENDIX.

In two cases where there was a marked difference in fertility in Experiments 2A and 2B, viz. at the 3rd hour and the 18th hour after the onset of oestrus the observations were repeated on two additional groups of 10 sheep. A further observation on ten sheep served 45 hours after the onset of oestrus was made. The data are summarized in the following tables:—

TABLE 3A (EXPERIMENT 2B).

GROUP 16.—EWES SERVED 3 HOURS AFTER THE ONSET OF OESTRUS.

Ewe No.	Occurrence of Oestrus.		Service 4 times.		Date Killed.	Result.
	Date.	Time.	Date.	Time.		
0230	1.2.32	7 a.m.	1.2.32	10 a.m.	25.4.32	+
18693	4.2.32	3 p.m.	4.2.32	6 p.m.	15.3.32	+
0238	5.2.32	7 a.m.	5.2.32	10 a.m.	15.4.32	+
M.B.A.	6.2.32	9 a.m.	6.2.32	12 noon	21.3.32	+
0243	7.2.32	1 p.m.	7.2.32	4 p.m.	14.3.32	+
0247	8.2.32	8 a.m.	8.2.32	11 a.m.	17.3.32	+
0253	10.2.32	7 a.m.	10.2.32	10 a.m.	17.3.32	+
0254	10.2.32	7 a.m.	10.2.32	10 a.m.	21.3.32	+
0263	12.2.32	10 a.m.	12.2.32	1 p.m.	18.3.32	+
0264	13.2.32	7 a.m.	13.2.32	10 a.m.	16.3.32	+

TABLE 8A (EXPERIMENT 2B).

GROUP 17.—EWES SERVED 18 HOURS AFTER THE ONSET OF OESTRUS.

Ewe No.	Occurrence of Oestrus.		Service 4 Times.		Date Killed.	Result.
	Date.	Time.	Date.	Time.		
0229	30.1.32	2 p.m.	31.1.32	8 a.m.	30.4.32	+
0234	2.2.32	12 noon	3.2.32	6 a.m.	23.4.32	+
0237	4.2.32	1 p.m.	5.2.32	7 a.m.	5.4.32	+
0244	5.2.32	4 p.m.	6.2.32	10 a.m.	14.3.32	+
0256	9.2.32	1 p.m.	10.2.32	7 a.m.	31.3.32	+
0255	10.2.32	1 p.m.	11.2.32	7 a.m.	21.3.32	+
0258	11.2.32	3 p.m.	12.2.32	9 a.m.	16.3.32	+
0251	11.2.32	5 p.m.	12.2.32	11 a.m.	16.3.32	+
B 26	13.2.32	11 a.m.	14.2.32	5 a.m.	14.3.32	+
0266	13.2.32	3 p.m.	14.2.32	9 a.m.	14.3.32	+

VITALITY OF SPERMATOZOAN OF MERINO SHEEP.

TABLE 16A (EXPERIMENT 2B.).

GROUP 18.—EWES SERVED 45 HOURS AFTER THE ONSET OF OESTRUS.

Ewe No.	Occurrence of Oestrus.		Service 4 Times.		Recurrence of Oestrus.	Date Killed.	Result.
	Date.	Time.	Date.	Time.			
0233	30.1.32	12 noon	1.2.32	9 a.m.	16.2.32	—	—
*0231	1.2.32	5 p.m.	3.2.32	2 p.m.	21.2.32	—	—
0209	4.2.32	6 p.m.	6.2.32	3 p.m.	22.2.32	—	—
0240	5.2.32	3 p.m.	7.2.32	12 noon	22.2.32	—	—
0241	6.2.32	9 a.m.	8.2.32	6 a.m.	23.2.32	—	—
0242	6.2.32	9 a.m.	8.2.32	6 a.m.	—	16.3.32	+
0144	10.2.32	6 p.m.	12.2.32	3 p.m.	28.2.32	—	—
0169	11.2.32	11 a.m.	13.2.32	8 a.m.	29.2.32	—	—
0265	12.2.32	9 a.m.	14.2.32	6 a.m.	1.3.32	—	—
0260	12.2.32	10 a.m.	14.2.32	7 a.m.	—	15.3.32	—

TABLE 16B (EXPERIMENT 2B.).

RESUME OF TABLES 3A, 8A, and 16A (EXPERIMENT 2B.)

Group No.	Time of Service After Onset of Oestrus.	Number Served.	Percentage Fertility.
16	3 hours	10	100
17	18 hours	10	100
18	45 hours	10	10

NOTE.—The ten ewes served at 45 hours after the onset of oestrus behaved as follows to the rams: 6 refused service altogether and had to be held: 1 accepted service from two rams and refused the third, and 3 definitely stood for the rams. 0242, the only ewe pregnant refused the rams.

* This ewe was served on the 3rd February. She lambed on the 2nd July, after a gestation period of 150 days. The lamb was normal weighing 7·2 lb. She showed a recurrence of oestrus on the 21st February (i.e. 19 days following the date of service), on which date she was artificially inseminated in another experiment. Obviously fertilization could not have taken place following artificial insemination, since in that case gestation would have only been 132 days. The shortest normal gestation period recorded by us is 142 days. It would therefore appear that this ewe showed external manifestations of oestrus, allowing copulation with the teaser, even though she was actually pregnant to the service on the 3rd February.

Section VII.

Poisonous Plants.

- D. G. STEYN ... Investigations into the Toxicity of Known and Unknown Poisonous Plants in the Union of South Africa.
- D. G. STEYN ... *Chrysocoma tenuifolia* Berg. Poisoning in Angora Goats and the Development of Tolerance.
- D. G. STEYN ... A Study of the Factors concerned in the Determination of the Toxicity of *Cotyledon orbiculata*.
- D. G. STEYN ... Experiments with Potassium Cyanide on Rabbits.
- D. G. STEYN AND G. DE KOCK. Crotalariosis in Sheep.
- CLAUDE RIMINGTON. Isolation and Chemical Examination of the Poisonous Principles of *Dimorphotheca spectabilis* Schltr. and *Dimorphotheca Zeyheri* Sond.

Investigations into the Toxicity of Known and Unknown Poisonous Plants in the Union of South Africa.

By D. G. STEYN, B.Sc., Dr.Med.Vet., Veterinary Research Officer,
Onderstepoort.

(Continued from the 17th Report of the Director of
Veterinary Services and Animal Industry.)

AIZOACEAE.

Mesembrianthemum savicolon N.R.Br. [Spec. No. 2223 (b);
Iron peg No. 147.]

Common name: Klipvygie.

Origin: Constantia, Rouxville, O.F.S.

State and stage of development: Fresh and in the pre-flowering stage.

This plant was suspected to have caused mortality in stock.

Rabbit: 30 grams of the fresh leaves per stomach tube at 2 p.m.
on 24.7.31.

Result: Negative.

APOCYNACEAE.

Vinca major L. (Spec. 4776; 1/10/30).

Common name: Periwinkle; maagdepalm.

Origin: Skilpadbeen, Willowmore.

State and stage of development: This plant was grown at Onderstepoort and tested in a fresh state and in the flowering stage.

The owner of the farm Skilpadbeen, having lost some calves which had been running under trees where this plant grew luxuriantly, suspected it of having caused the deaths.

Sheep 29669: 600 grams of the fresh young shoots, leaves and flowers per stomach tube at 12 noon and another 600 grams at 3.30 p.m. on 18.9.31.

Result: Negative.

Three rabbits dosed with 10 grams, 30 grams and 60 grams respectively, developed no symptoms of ill-health.

Vinca major L. is officially known as *Perwenche officinale* in the French Pharmacopœia. It has been used as an astringent as it contains a certain amount of tannic acid.

As claims have been made with regard to the value of this plant in the treatment of *diabetes mellitus*, Epstein (1926) submitted it to some tests in order to ascertain the value of these claims. The results of his investigations are summed up as follows:—

“Covinca, or vinca, has no effect (a) in reducing the normal or fasting blood sugar of rabbits, nor has it any effect on (b) experimental hyperglycaemia produced in rabbits by adrenalin.

It has a very weak digitalis-like action on the circulatory system.”

Messrs. Lennon Ltd. of South Africa is marketing under the name of “Covinca” a preparation of *Vinca major*.

Nye and Fitzgerald of Australia (Epstein 1930), who have tested the effects of a tincture of the vinca plant on diabetic patients came to the following conclusions:—“Vinca produces no appreciable effect on the fasting sugar content of the blood or urine in patients with diabetes, . . . nor does it diminish the rise of the curve after glucose.”

ARACEAE.

Colocasia antiquorum Schott (Spec. No. 4557).

Origin: Pretoria North.

Common name: Elephant ear.

State and stage of development: Fresh and in the post-flowering stage.

Pammel in his Manual of Poisonous Plants mentions this plant as an acrid poison. It is a cultivated garden plant.

A child was reported to have chewed a piece of the leaf, with the result that the tongue was severely burnt.

Rabbit: 30 grams of the fresh leaves per stomach tube at 4 p.m. on 30.11.31.

Result: Negative.

In discussing the above plant in his Flowering Plants and Ferns, Willis says:—“Cultivated for its rhizomes, which when boiled lose their poisonous nature and form a valuable foodstuff known as “Taro”, “Coco” and “Eddoes”.

CAPPARIDACEAE.

Cadaba juncea (L.) Benth. and Hook., F. [Spec. No. 3277 (b)].

Common name: Swartstorm.

Origin: M. J. Latsky, Rietvlei, Carnarvon.

State and stage of development: Dry and no flowers or fruits present.

Rabbit: 30 grams of the dry plant per os on 29.9.31.

Result: Negative.

COMPOSITAE.

Aster filifolius Auct. non Vent. [Spec. No. 2223 (a)].

Common name: Draaibos.

Origin: "Constantia", Rouxville.

State and stage of development: Fresh and in the early flowering stage.

Two specimens of the above plant were submitted by the Government Veterinary Officer, Aliwal North, with the remark that he suspected it of having caused death in two hundred sheep within two days on the farm "Constantia". The deaths had occurred amongst sheep which had been moved to Constantia from another farm, where this plant does not occur. The post-mortem symptoms described were:—Intestines filled with coagulated blood, which was passed out to some extent before death; liver and spleen were slightly enlarged and showed marked hyperaemia; the rumen contained a large amount of "draaibos".

It is of interest to note that the owner has never lost any of the sheep which were acquainted with the vegetation on the farm. Many farmers regard the "draaibos" as a valuable feed, especially in the lambing season.

This plant was also reported on in the previous report of the Director of Veterinary Services.

As very little material was available, only one rabbit could be dosed.

Rabbit: 40 grams of the fresh leaves and buds at 2 p.m. on 24.7.31. 25.7.31: Not feeding; another 25 grams of fresh leaves and buds. Within one hour after dosage pronounced laboured respiration set in.

26.7.31: Died previous night.

Post-mortem appearances: Dilatation of right ventricle, which contained a large amount of coagulated blood; slightly hyperaemia and pronounced oedema of lungs.

CRASSULACEAE.

Cotyledon decussata Sims. [Spec. No. 3277 (c); Iron peg 105].

Common name: Plakkies.

Origin: M. J. Latsky, Rietvlei, Carnarvon.

State and stage of development: Fresh and just budding.

Rabbit: 100 grams of the fresh leaves per os on 23.9.31.

Result: 24.9.31. Nothing unusual; another 50 grams of fresh leaves. Within half an hour after dosage there was weakness of the neck, the animal being unable to keep the head up. This condition progressed until the animal was unable to sit up. The respiration was superficial and hurried and the heart-beat accelerated and weak.

25.9.31. Condition as above; paralysis progressing.

26.9.31. Completely paralysed, pronounced dyspnoea; heart-beat almost imperceptible.

27.9.31. Ditto.

28.9.31. Died in a state of complete paralysis.

Post-mortem appearances: The urinary bladder was strikingly distended with normal urine; otherwise nothing unusual was noticed.

A 96 per cent. alcoholic extract of the leaves was prepared and injected subcutaneously into guinea-pigs in quantities of 5, 10, 30, 50 and 100 grams respectively. The attacks of convulsions, which are so typical of *Cotyledonosis* in guinea-pigs, were developed within one and a half to quarter of an hour after injection respectively. All the animals died in a state of complete paralysis within thirty hours to half an hour after injection respectively.

Curson was the first to produce experimentally chronic cases of "krimpsiekte" by feeding goats on *Cotyledon wallichii*. (See Curson's Report to Acting Director of Veterinary Education and Research, Pretoria, 6th October, 1920, File 34, Grahamstown.)

An interesting observation made by Curson is the fact that kraaled goats do not develop the typical symptoms of "krimpsiekte", which are precipitated when the animals are driven.

This point is of the utmost importance in many poisonous plants tested under laboratory conditions where the animals are confined to small boxes.

CUCURBITACEAE.

Cucumis naudinianus Sond. (Spec. No. A; 20/5/31).

Common name: Agurkie, wilde komkommer, wild cucumber.

Origin: Near Pienaars River, Transvaal.

State and stage of development: fresh ripe fruit.

Rabbit: received per stomach tube 85 grams of the fresh ripe fruit.

Result: Negative.

EQUISETACEAE.

Equisetum ramossissimum Desf. (Spec. No. 1311; 2/6/31).

Common name: Dronkgras, drilgras.

Origin: C. J. G. Loock, Lissie, P.O. Slabberts Station, Orange Free State.

State and stage of development: Mouldy and in pre-flowering stage.

The following information in regard to the toxicity of this plant was supplied by Mr. Loock. When sheep have eaten this plant and are driven, they suddenly stop, stand with their heads hanging, while their bodies appear to be itchy. If the driving is stopped, the animal will lie down or stand with arched back and outspread legs. Sometimes the affected animals kick as if there is pain in the abdomen.

If such affected animals are urged for about four or five hundred yards they ultimately go down, and most of them die immediately or within a few hours. He adds that death invariably occurs if affected animals are driven.

Sheep 20778 and 21742 received 2.25 and 9 kilograms of the dried plant in the course of seventeen days respectively.

Result: On the tenth day of the experiment these two sheep with two control sheep were driven about for half an hour. Sheep 20778 and 21742 tired very quickly and were exhausted, whereas the two control sheep hardly exhibited any symptoms of dyspnoea. In addition, the two controls showed normal breathing within a few minutes after the discontinuation of the driving, whereas the two experimental animals were still panting after ten minutes.

On the last day of the experiment the above test was repeated with identical results. At no time were any muscular spasms seen.

Isolation of the toxin: Very little plant material was available. Chloroform, absolute alcohol, 50 per cent. alcohol, ether and aqueous extracts were injected subcutaneously into guinea-pigs.

Within six hours after the injection of the evaporated absolute alcoholic extract the guinea-pigs exhibited convulsions of the whole body. These convulsions were precipitated when the animal was excited. It died within thirty-six hours of the injection.

Post-mortem appearances: Heart in diastole and both ventricles distended with coagulated blood; the subcutaneous tissues surrounding the point of injection were slightly infiltrated by a colourless gelatinous material. Owing to lack of material this investigation could not be continued.

Discussion: It is possible that the plant in a mouldy state is less poisonous than the fresh plant.

EUPHORBACEAE.

Euphorbia helioscopia L.

Common names: Melkgras, melkbos, wolwemelk, milk weed, spurge.

Origin: Poisonous-plant garden, Onderstepoort. The plants were grown from seed collected from a specimen which had been forwarded from Capetown. (Spec. No. 5179; 8/10/30.)

State and stage of development: Fresh and in the late flowering stage.

Sheep 26654: Received per stomach tube 1,000 grams of the whole plant on 27.2.31.

28.2.31: Another 1,000 grams.

Result: Negative.

Sheep 23555: Received per stomach tube 2,100 grams of the whole plant in the pre-flowering stage within twenty-four hours.

Result: Negative.

Euphorbia mauritanica L. [Spec. 3277 (b)].

Common name: Vingerpol; geel melkbos.

Origin: M. J. Latsky, Rietvlei, Carnarvon.

State and stage of development: Fresh and flowering.

Rabbit: 20 grams of the above fresh plant per os on 23.9.31.

Result: Negative.

This plant was suspected of having caused mortality in sheep.

LEGUMINOSAE.

Crotalaria nubica Benth. (Spec. No. 569; 9/5/31).

Common name: ———.

Origin: Mr. Blignaut, Whitehaven, P/B Stockpoort, via Nylstroom.

State and stage of development: Fairly mouldy and in the late seeding stage.

Bovine 4199 (11 months old) ingested 50 kilograms of the dried plant in forty days.

Result: Negative.

Sheep 29669 and 29652 received per stomach tube 2·7 and 10·8 kilograms of the dried plant in the course of 21 days respectively.

Result: Negative.

Lessertia physodes E. and Z.

(N.H. No. 10051.)

Common name: ———.*Origin*: The seeds were obtained from East London and sown in the poisonous-plant garden at Onderstepoort.*State and stage of development*: Fresh and in the late flowering stage.*Rabbit*: 50 grams of the above fresh leaves, flowers and immature fruit per os on 22.9.31.*Result*: Negative.

LILIACEAE.

Albuca fastigiata Dryand (N.H. No. 10060). [Spec. No. 6948 (*b*); Iron peg 106.]*Common name*: ———.*Origin*: "Rocklands", P.O. Klipdam, Cape Province.*State and stage of development*: Fresh and in flowering stage. This plant was suspected of having caused mortality in sheep and cattle.*Rabbit*: 70 grams of the fresh bulbs, leaves and flowers per stomach tube at 4 p.m. on 6.1.31.*Result*: Negative.*Albuca altissima* Dryand (N.H. No. 10058). [Spec. No. 6948 (*a*); Iron peg 54.]*Common name*: ———.*Origin*: "Rocklands", P.O. Klipdam, Cape Province.*State and stage of development*: Fresh and in the pre-flowering stage. This plant was alleged to have caused death in sheep and cattle.*Rabbit*: 150 grams of the fresh bulb and leaves per stomach tube at 4 p.m. on 6.1.31.*Result*: 7.1.31—died previous night.*Post-mortem appearances*: Acute catarrhal gastritis with pronounced hyperaemia; rupture of the stomach.

As it was doubtful whether the plant was the cause of death in this rabbit, another rabbit and a sheep were dosed.

Rabbit: 100 grams of the fresh bulb and leaves at 11 a.m. on 7.1.31.*Result*: Negative.

Sheep 21742: 500 grams of the fresh bulbs and leaves at 12 noon on 19.1.31.

20.1.31—apparently healthy; another 500 grams.

Result: Negative.

Bulbine narcissacfolia Salm-Dyck. [Spec. 6948 (c).]

Common name: ———.

Origin: J. F. de Villiers, "Rocklands", P.O. Klipdam, Cape Province.

State and stage of development: Fresh and in the early seeding stage. Mr. de Villiers suspected this plant of having caused mortality in his sheep and cattle.

Sheep 23498: Received per stomach tube 500 grams of the above fresh bulbs and leaves on 19.1.31. 20.1.31: another 500 grams.

Result: Negative.

Dipeadi glaucum Bkr. (N.H. No. 10129). (Spec. 7715.)

Common name: Malkop-ui; wild onion.

Origin: G. G. Wayland, Fort Richmond, P.O. Belmont, Kimberley.

State and stage of development: Fresh and in the early flowering stage.

Sheep 23741: Received per stomach tube the following quantities of the fresh bulbs, leaves, flowers and flower stalks:—

18.2.31: 100 grams; 19.2.31: 100 grams; 20.2.31: 200 grams; 21.2.31: 300 grams.

No more plant material was left. On 25.2.31 a further supply of the fresh plant in the late flowering stage was received. The above sheep was then drenched with the whole plant as follows:—

26.2.31: 300 grams; 27.2.31: 300 grams; 28.2.31: 500 grams; 1.3.31: 900 grams.

Result: From 6.3.31 the animal exhibited the following symptoms:—laboured respiration; accelerated and weak pulse; diarrhoea; fever; apathy; standing with head in corner; unwilling to move; and lying down frequently. Death occurred during the night of 11.3.31.

Post-mortem appearances: Marked hydropericardium and an acute catarrhal gastro-enteritis.

Sheep 24024: Received per stomach tube the whole plant in the fresh state in the following quantities:—

18.2.31: 500 grams; 19.2.31: 800 grams; 20.2.31: 900 grams; 21.2.31: 900 grams; no more plant material left. A further supply of the fresh plant in the late flowering stage was received on 25.2.31.

This animal was then drenched with the whole plant in a fresh state as follows:—26.2.31: 900 grams; 27.2.31: 900 grams; 28.2.31: 900 grams; at 3 p.m., 28.2.31, a slight diarrhoea was noticed; 1.3.31: diarrhoea; fever; apathetic; accelerated and weak pulse and laboured respiration.

2.3.31. Died previous night.

Post-mortem appearances: The advanced state of decomposition obscured all lesions.

Sheep 29509: Received per stomach tube the following quantities of the fresh whole plant in the late seeding stage.

14.4.31: 690 grams; 15.4.31: apathetic; 700 grams; 16.4.31: slight diarrhoea; apathetic, laboured respiration, accelerated pulse; fever (temp. 105° F.).

17.4.31: Condition as on 16.4.31; animal pushing its head in a corner of the stable and remaining in this position for hours (temp. 105.6° F.).

18.4.31: Condition same, still pushing in the corner of the stable; pulse weak and accelerated; pronounced cyanosis (temp. 106° F.).

19.4.31. Died.

Post-mortem appearances: Pronounced general cyanosis; congestion of the lungs; degenerative changes in the heart, liver and kidneys; subepicardial and subendocardial haemorrhages; calcified nodules in the lungs; hyperaemia and swelling of the lymph-glands; hyperaemia of the gastro-intestinal mucosa.

As many farmers complained of heavy losses among their stock (cattle, sheep and goats) and rarely horses, due to this plant causing abortion, it was decided to conduct some experiments with pregnant ewes. Six ewes in the fourth month of pregnancy were utilized in these experiments.

In these experiments the fresh bulb in the post-seeding stage obtained from Postmasburg was utilized.

A rabbit drenched with 75 grams of the fresh bulb developed the following symptoms:—Emesis within five minutes after dosing; weakness (paresis) of the front quarters set in and progressed until the animal was completely paralysed; no cornea reflex; extreme dyspnoea; accelerated and weak pulse; death supervened within one and three quarter hours after dosage.

Post-mortem appearances: Plant material in the bronchi and trachea; cyanosis; pronounced hyperaemia and oedema of the lungs; hyperaemia of the gastric mucosa.

The results of the experiments with pregnant ewes are contained in the following table:—

THE EFFECTS OF *DIPLOCLADIA GLAUCUM* ON PREGNANT EWES.

No. of pregnant ewe.	Date of dosage and quantity of plant given.	Result.
21430	22.6.31—600 grams.... 23.6.31—600 grams....	23.6.31—not feeding, apathetic, temp. 105.4° F. 24.6.31—very apathetic; swaying from side to side; swaying gait; pushing hard in corner of stable and supporting the body against water trough; pulse 164 and weak; laboured respiration; cyanosis; pronounced bloody diarrhoea. temp. 108° F. 25.6.31—died previous night. <i>Post-mortem appearances</i> : Pronounced general cyanosis; blood dark-red and tarry in consistence; pronounced hyperaemia and slight oedema of the lungs; severe acute catarrhal gastro-enteritis; uterus contained a 4½ months old dead foetus.
21623	22.6.31—600 grams.... 23.6.31—600 grams....	23.6.31—not feeding, apathetic, temperature 105° F. 24.6.31 and 25.6.31—symptoms very similar to those exhibited by ewe 21430. 25.6.31—condition worse; temperature 105.8° F. 26.6.31—died previous night. <i>Post-mortem appearances</i> : Intense general cyanosis; ruptured foetal membranes protruding from vulva; pronounced hyperaemia and slight oedema of the lungs; numerous subpericardial haemorrhages; degenerative changes in the myocard and liver; very pronounced acute catarrhal gastro-enteritis; 4½ months old dead foetus in uterus.
13093	22.6.31—300 grams.... 23.6.31—300 grams.... 24.6.31—300 grams....	23.6.31—not feeding; apathetic; temperature 103.8° F. 24.6.31—symptoms very similar to those exhibited by ewe 21430 only less severe; temperature 105.8° F. 25.6.31—died previous night. <i>Post-mortem appearances</i> : General cyanosis; ruptured foetal membranes protruding from vulva; hyperaemia and oedema of the lungs; subpericardial haemorrhages; degenerative changes in the myocard and liver; pronounced acute catarrhal gastro-enteritis; 4½ months old dead foetus in uterus.
21446	22.6.31—300 grams.... 23.6.31—300 grams.... 24.6.31—300 grams....	The symptoms coincided with those exhibited by ewe 13093, death occurring on the night of 24.6.31. The post-mortem appearances were also very similar to those of ewe 13093 with the exception that ewe 21446 showed a severe haemorrhagic gastro-enteritis. Also in this case the ruptured foetal membranes protruded from the vulva.
21443	From 25.6.31 to 27.6.31 animal received 300 grams of plant at the rate of 100 grams daily	26.6.31—inappetence, listless. 27.6.31—inappetence, diarrhoea; apathetic; pulse and respiration accelerated; pulse accelerated and weak; laboured respiration 39.6.31—condition as on previous day; pressing head in corner of stable. 1.7.31—aborted at 7 a.m.; condition worse. 2.7.31—lying prostrate, unable to rise; temperature 105.4° F.; profuse foetid diarrhoea; pulse extremely weak and accelerated; cyanosis. <i>Post-mortem appearances</i> : Intense general cyanosis; marked hyperaemia of lungs with multiple localised broncho-pneumonic areas and necrotic area in left central lobe; haemorrhages in mediastinal and bronchial lymph-glands; haemorrhages on mucous membranes of urinary bladder; haemorrhages in spleen; retention of necrotic foetal membranes; pronounced acute catarrhal gastro-enteritis with numerous haemorrhages in caecum and colon.
24235	From 25.6.31 to 27.6.31 animal received 300 grams of plant at the rate of 100 grams daily	26.6.31—inappetence; listlessness. 27.6.31—inappetence; diarrhoea; apathetic; pulse accelerated but strong; dysnoea. 28.6.31—condition worse; diarrhoea. 29.6.31—condition worse, lying down most of the time. 30.6.31 as on 29.6.31. 1.7.31—pressing head against stable wall, very weak, pulse weak and accelerated, pronounced dyspnoea, profuse diarrhoea. 2.7.31—worse; unable to rise, temperature 105.4° F.; died at 11 a.m. <i>Post-mortem appearances</i> : General cyanosis; hyperaemia of lungs; hyperaemia, degeneration and pigmentation of liver; extensive haemorrhage in spleen; haemorrhages in retropharyngeal and mediastinal lymph-glands; pronounced acute catarrhal gastro-enteritis with extensive ulceration of the abomasal mucous membrane; partly macerated 4½ months old foetus in uterus.

From the above table it is evident that the bulbs of *Dipcadi glaucum* contain a severe gastro-intestinal irritant. The most outstanding symptoms produced by this plant in pregnant Merino ewes were:—profuse diarrhoea, extreme weakness, pressing into the corners or against the wall of the stable, high fever, accelerated and weak pulse and dyspnoea. Furthermore, it caused death and in some cases partial or complete expulsion of the foetus.

The death and expulsion of the foetus are caused most probably indirectly through the severe gastro-intestinal irritation. It is, however, possible that the plant poison, after absorption into the system, and harmful substances which pass through the damaged gastro-intestinal mucosa, have a detrimental effect on the pregnant uterus and its foetus.

Discussion: From all over Griqualand West reports of heavy losses amongst cattle, sheep and goats were received. Farmers describe the symptoms as follows:—Affected animals abort or give birth to weak young; they go raving mad and never lie down but walk and walk until they collapse and die; when given a fright they will run for miles; they jam themselves in bushes or fences and will remain in that position for days. The only post-mortem lesions described is an acute inflammation of the gastro-intestinal tract.

The following is an extract from a letter received from Mr. Wayland:—

“The wild onion has been known on this farm and many other farms in this district since 1867, and its poisonous effect on cattle, sheep, goats and pigs. Losses have been sustained from this plant for many years back, especially in pregnant stock, in which case abortion has accounted for a big loss in the increase. The wild onion grows only in sand veld, usually on the west side of hills. It grows with the first rains that fall in December or January. If rains are late the onion is late, but always poisonous. The Vermin Proof Act was enforced in this district in June, 1929. This farm with some internal camps was completed on November 8th, 1930, when all sheep were allowed to run day and night. I was told by an old farmer in this area that sheep running free were not affected by the onion. This has definitely been proved not to be the case. One of the camps into which sheep were put has a lot of wild onion in it. I took the risk, and after the first rains which fell in January, 1931, the onion came up thick and fast, sheep, cattle and goats taking to the onion as usual, with the result that losses up to date are: 132 sheep (Merinos), 9 have recovered. The sheep were removed at once; no more cases have occurred since their removal to veld free from wild onion. In cattle, 9 have died (adults); 2 calves, 4 aborted, 1 calf has recovered. In goats, 3 have died, and 1 kid aborted. The onion affects all ages and both sexes in the stock mentioned.

This year the onion has been more severe than known in former years in the case of cattle, and probably due to the lack of sufficient other grazing owing to the severe drought during the whole of 1930. Before netting the farms, sheep were always herded away from the onion and where they did get hold of it, it never failed to cause abortion even though the sheep (ewes) did not get sick themselves.

Our chief lambing season is from March to June, and the most calves fall from January into the winter. The wild onion does not die until the first frosts kills it, often as late as May, and grows during the gestation period of the ewes and some of the cows."

The cases produced at Onderstepoort by drenching non-pregnant sheep with *Dipcadi glaucum* appear to be similar to those described by farmers as occurring under natural conditions.

Dipcadi sp. (Probably undescribed) (N.H. No. 10061.) [Spec. No. 6948 (*d*).]

Common name: ———.

Origin: Rocklands, P.O. Klipdam, Cape Province.

State and stage of development: Fresh and in the pre-flowering stage. This plant was alleged to have caused losses in stock.

Rabbit: 100 grams of the fresh bulbs and leaves per os at 3 p.m. on 19.1.31.

Result: Negative.

Scilla lanceaeifolia Bkr. [Spec. No. 6948 (*e*); Iron peg 119].

Common name: wild squill.

Origin: Rocklands, P.O. Klipdam, Cape Province.

State and stage of development: Fresh and in the flowering stage. This plant was suspected of having caused mortality in sheep and cattle.

Rabbit: 100 grams of the fresh bulbs, leaves and flowers per stomach tube at 4 p.m. on 6.1.31.

Result: Negative.

Urginea altissima Bkr. (N.H. No. 10057). (Spec. No. 26.)

Common name: Maarman.

Origin: Goss Estates, Vryheid, Natal.

State and stage of development: Fresh and in the post-flowering stage. Calves were suspected to have died from this plant.

Sheep 26654: 500 grams of the fresh bulbs and leaves per stomach tube at 12 noon 7.4.31.

Result: 8.4.31. Apathetic, not feeding; another 500 grams.

9.4.31. Apathetic, inappetence; accelerated and weak pulse; laboured respiration.

10.4.31. Died previous night.

Post-mortem appearances: Pronounced general cyanosis; both ventricles of the heart markedly dilated; degenerative changes in the myocard; pronounced hyperaemia and doedema of the lungs with

numerous haemorrhages at the bifurcation of the trachea; oedema of and haemorrhages in the mediastinal lymph-glands; slight subacute catarrhal duodenitis and jejunitis.

Sheep 29130: 200 grams of the fresh bulb and leaves per stomach tube at 11 a.m. on 10.4.31.

Result: 11.4.31. Apparently healthy; another 200 grams.

12.4.31. Apathetic; inappetence.

13.4.31. Laboured respiration; apathetic; inappetence; another 300 grams.

14.4.31. Condition as on 13.4.31; another 300 grams.

15.4.31. Cyanosis; laboured respiration; accelerated and weak pulse; apathetic; inappetence.

16.4.31. Died previous night.

Post-mortem appearances: 'Marked general cyanosis; subcutaneous blood-vessels markedly distended with dark reddish tar-like blood; hyperaemia of the lungs; hyperaemia of and haemorrhages in the mediastinal lymph-glands; acute catarrhal enteritis.

Histology: Liver: Extensive diffuse hyperaemia affecting the whole lobule uniformly and especially the vessels in the periphery.

Kidney: Hyperaemia.

Natives use the bulbs of this plant to prevent dogs from damaging skins which are laid out to dry. The bulbs are cut into halves and the cut surfaces rubbed on to the under surface of the skin, and it is said that dogs licking a skin thus treated vomit profusely.

SCROPHULARIACEAE.

Nemesia capensis O. Kuntze. (Spec. No. AB.)

Common name: Wit leebekkie.

Origin: The plants utilized in this experiment were obtained from seeds collected at Uniondale and sowed at Onderstepoort.

State and stage of development: Fresh and in the flowering stage. Farmers in the Uniondale District hold this to be an extremely poisonous plant.

Sheep 29652: 600 grams of the fresh stems, leaves and flowers per stomach tube at 12 noon and another 600 grams at 3.30 p.m. on 18.9.31.

Result: Negative.

Sheep 24692: Received 300 grams of the above dry plant in the late seeding stage on each of five consecutive days.

Result: Negative.

SOLANACEAE.

Cestrum laevigatum Schltr. (Spec. No. 8177.)

Common name: Inkberry bush.

Origin: East London.

State and stage of development: Wilted and in the flowering and fruiting stage (green and ripe fruits).

Sheep 21742: Received per stomach tube 2,000 grams of the wilted and dry whole plant (stems, leaves, flowers, green and ripe fruit) in the course of eight consecutive days.

Result: Negative.

Sheep 29509: Received per stomach tube 3,200 grams of the wilted and dry whole plant in the course of eight consecutive days. Subsequently this animal received 4 kilograms of the fresh, wilted and dry leaves of this plant in the pre-flowering stage.

Result: Negative.

Rabbit: 30 grams of the fresh green berries per stomach tube.

Rabbit: 30 grams of the fresh ripe berries per stomach tube.

Result: Negative.

Nicotiana glauca R. Grah.

Common name: Wild tobacco.

Origin: Poisonous-plant garden, Onderstepoort. (Seeds were collected on the farm Skilpadbeen, Willowmore, and planted at Onderstepoort.)

State and stage of development: Fresh and in the flowering stage.

Farmers are unanimous in their reports that wild tobacco is a deadly poison to ostriches. Hutcheon (1903) reported that wild tobacco is poisonous to cattle, sheep and ostriches, and that a farmer of Bloemfontein had lost five oxen which had partaken of this plant. In the same report Hutcheon mentions that two transport riders had lost twenty-one out of twenty-eight oxen from wild tobacco.

Experiments were conducted with rabbits to ascertain the relative toxicity of the flowers and leaves, and it was found that the former were slightly less toxic than the leaves. 30 grams of the fresh flowers caused death in rabbits within one and a half hours, while 30 grams of the fresh leaves produced death within half an hour after dosage. Again 20 grams of the fresh flowers failed to produce any symptoms of poisoning in a rabbit, while a rabbit dosed with 20 grams of the fresh leaves exhibited pronounced symptoms of poisoning but recovered within twenty-four hours.

The symptoms and post-mortem appearances produced by the flowers and leaves were identical and could be summarised as follows:—Within five to thirty minutes after dosage (depending on

the size of the dose) dyspnoea set in. Attacks of clonic spasms of all the body muscles were exhibited. These attacks, which at the beginning were not very severe and lasted only a few seconds, became progressively pronounced and occurred at shorter intervals until ultimately they were continuous. The dyspnoea became so pronounced that the animals gasped for breath. On account of the convulsions it was impossible to control the heart-beat. There was pronounced protusion of the membrana nictitans during the attacks of convulsions and at the point of death. After death the quivering of the muscles of mastication continued for quite a few minutes. At the point of death the heart-beat could not be felt. Paralysis set in in the front quarters and progressed until the whole body was paralysed.

Death occurred with convulsions and symptoms of asphyxia within a quarter of an hour to one and a half hours after dosage.

Post-mortem appearances: General cyanosis; pronounced hyperaemia of the lungs; marked dilatation of both ventricles of the heart.

Sheep 21742: This animal received 300 grams of the fresh leaves per stomach tube. Within half an hour of dosing the animal showed depression, unwillingness to move and when driven exhibited marked ataxy, the steps were very short and the animal had great difficulty in maintaining its balance. Urging caused the precipitation of clonic spasms of all body muscles. In addition there was grinding of the teeth and groaning, while dyspnoea was marked. The symptoms became progressively severe until one and a half hours after dosage the animal was prostrate and unable to rise. At varying intervals clonic spasms of the muscles of the fore- and hind-quarters occurred. Salivation was increased and rales could be heard over the trachea and larynx. The pulse was 180 per minute and very weak.

The animal died in a comatose state and with symptoms of asphyxia within two and a half hours after dosage.

Post-mortem appearances: General cyanosis; pronounced hyperaemia of the subcutaneous tissues; subepicardial haemorrhages; extensive haemorrhage into the right lung with hyperaemia and oedema; hyperaemia of the liver; haemorrhages in the duodenal mucous membrane.

Solanum incanum Linn.

Common name: Bitter apple.

Origin: Poisonous-plant garden, Onderstepoort.

State and stage of development: Fresh and in the flowering and early fruiting stage. The green fruits utilised in this experiment measured from 1 to 3 cm. in diameter.

1 *rabbit:* Received per stomach tube 40 grams of the fresh green (unripe) fruit at 3 p.m. on 24.3.31.

Result: 25.3.31: 7.30 a.m.: Animal appears ill, moves with difficulty, apathetic, not feeding, heart-beat accelerated and respiration laboured. It was decided to give the animal another 40 grams of the green fruit and, as the stomach tube was being introduced the animal struggled, made a few gasps and died.

Post-mortem appearances: Dilatation of both ventricles of the heart; and a very severe acute catarrhal gastritis with large haemorrhagic patches on the mucous membrane.

1 rabbit: Received per stomach tube 50 grams of the fresh green fruit at 4 p.m. on 26.3.31.

Result: 27.3.31. 7.30 a.m.: Listless; not feeding. Another 50 grams of the fresh fruit at 12.30 p.m. Within half an hour after dosage the animal showed restlessness, an accelerated and weak heart, beat, ataxy, laboured respiration, and died with symptoms of asphyxia within two hours after dosage.

Post-mortem appearances: Marked emphysema of both lungs; pronounced dilatation of both ventricles of the heart; and an acute catarrhal gastritis.

Solanum nigrum L. (Iron peg 82; Spec. No. ABC.)

Common name: Black nightshade, galbessie, nastergal.

Native name: Msobamsoba.

Origin: School of Agriculture, Teko, Butterworth.

State and stage of development: Leaves slightly decomposed; in early fruiting stage.

Rabbit: Received 35 grams of the green berries.

Result: Died over night.

Post-mortem appearances: Both ventricles of heart distended with coagulated blood; pronounced hyperaemia of lungs and gastric mucosa.

The above plant material was forwarded by the Government Veterinary Officer, Butterworth, as it was suspected to have caused mortality in sheep grazing on lands where this plant grew very luxuriantly. The symptoms described were: sudden onset of pain and unsteady gait and death with convulsive movements.

Post-mortem appearances: Slight enteritis; slight degeneration of the liver; hyperaemia and degeneration of the spleen and kidneys; in some cases very pronounced nephritis affecting principally the cortex.

Solanum panduraeforme E. Mey. (Spec. AC.)

Common name: Bitter apple.

Origin: Collected in the vicinity of the Power Station, Onderstepoort.

State and stage of development: Fresh and in the flowering and early fruiting stage.

1 *rabbit*: Received per stomach tube 30 grams of the fresh green (unripe) fruit at 11 a.m. on 12.3.31.

Result: 13.3.31. 7.30 a.m. showed no ill-effects. Received another 30 grams of the fresh green fruit.

14.3.31. 7.30 a.m. Died previous night.

Post-mortem appearances: White froth exuding from nostrils and mouth; pronounced hyperaemia of the lungs; coccidiosis of the liver; and a very severe acute catarrhal gastro-enteritis.

1 *Rabbit*: Received per stomach tube 30 grams of the fresh green (unripe) fruits at 2.30 p.m. on 16.3.31.

Result: 17.3.31: 7.30 a.m. Shows no ill-effects. Received another 30 grams of the fresh green fruit.

4 p.m. Apathetic; accelerated heart-beat and respiration; and appears to be partly paralysed.

18.3.31. Died previous night.

Post-mortem appearances: General cyanosis; both ventricles of the heart distended with coagulated blood masses; pronounced hyperaemia of the lungs; and a very severe acute catarrhal gastro-enteritis with numerous haemorrhages on the gastric mucosa.

Solanum panduracforme E. Mey. (Spec. AD.)

Origin: Poisonous-plant garden, Onderstepoort.

State and stage of development: Fresh and in the flowering and early fruiting stage.

Green Fruit.

1 *Rabbit*: Received per stomach tube 40 grams of the fresh green (unripe) fruit at 3 p.m. on 24.3.31.

Result: 25.3.31: 7.30 a.m. Shows no ill-effects. Another 25 grams of the fresh green fruit.

26.3.31: 7.30 a.m. Apparently healthy; another 50 grams of the fresh green fruit. Within seven hours after dosage restlessness, inappetence, weak and accelerated pulse, laboured respiration and ataxy set in.

27.3.31: 7.30 a.m. Died previous night.

Post-mortem appearances: Dilatation of both ventricles of the heart; hyperaemia of the lungs; and a pronounced acute catarrhal gastritis.

Ripe Fruit.

1 *Rabbit*: Received per stomach tube 50 grams of fresh ripe fruit at 2 p.m. on 14.4.31.

Result: 15.4.31: 7.30 a.m. Apparently healthy. Received another 50 grams of fresh ripe fruit. Within four hours the animal exhibited restlessness and later dullness; progressive weakness until almost paralysed; laboured respiration and an accelerated and weak pulse. The animal died in a comatose condition ten hours after it had received the second dose.

Post-mortem appearances: Pronounced general cyanosis; marked dilatation of both ventricles of the heart; hyperaemia of the lungs and liver; and a pronounced acute catarrhal gastro-enteritis.

Solanum rigescens Jacq. (Spec. AE; 24.5.31.)

Common name: ———.

Origin: Poisonous-plant garden, Onderstepoort.

State and stage of development: Fresh and in the flowering and fruiting stage.

Unripe Fruit.

1 *Rabbit*: Received per stomach tube 40 grams of fresh green (unripe) fruit on 24.3.31.

Result: 25.3.31: Apparently healthy; another 40 grams.

27.3.31: Apparently healthy; another 40 grams.

Animal developed no symptoms of ill-health.

Ripe Fruit.

1 *Rabbit*: Received per stomach tube 40 grams of fresh ripe fruit on 24.3.31.

Result: 25.3.31: Apparently healthy; another 40 grams.

27.3.31: Apparently healthy; another 40 grams.

Animal developed no symptoms of ill-health.

THYMELAEACEAE.

Gnidia Burchellii Meisn. (Spec. 3788; 11.11.31.)

Common name: Repuisbossie; harpuisbossie.

Origin: Kuruman.

State and stage of development: Dry and in the flowering stage.

This plant abounds along the main road between Kuruman and Vryburg and farmers hold that it frequently is the cause of mortality in stock, especially in animals which are moved from one locality to another.

On grinding the dry plant the native boy experienced a feeling of severe burning in the nose, throat and chest, and dyspnoea, which progressed to such an extent that he had to be treated. Aspirators had to be used whenever the plant was weighed off for purposes of dosing the animals as the slightest amount of plant dust inhaled caused intense irritation of the air passages.

When a pinch of the ground plant was taken on the tongue a bitter taste was experienced. Even though the mouth was repeatedly rinsed out with water a "burning feeling" of the tip of the tongue and throat set in about five minutes after the material had been placed on the tip of the tongue and continued for quite a number of hours.

A small quantity of the ground plant was made into a paste with water and placed on the skin on the inside of the arm. No local irritation was set up within fifteen minutes of application.

Sheep 29669: 300 grams of the dried stems, leaves and flowers per stomach tube at 3.30 p.m. on 30.11.31.

Result: 1.12.31. Listless; lying down most of the time; laboured respiration and groaning on expiration; inappetence; accelerated and strong pulse.

2.12.31. Condition as on 1.12.31 + profuse diarrhoea.

3.12.31. Slight improvement; another 200 grams of plant given.

4.12.31. Listlessness; lying down; laboured respiration and groaning on expiration; inappetence; accelerated and weak pulse; profuse diarrhoea; bloated; salivation.

5.12.31. As on 4.12.31; temperature 105.8° F. Died at 3 p.m.

Post-mortem appearances: General cyanosis; pronounced hyperaemia and oedema of the lungs; acute catarrhal abomasitis with numerous haemorrhages; hydropericardium; hydroperitoneum.

DISCUSSION.

Biological tests were conducted with twenty-seven species of plants, namely *Mesembrianthemum sarricolum* N.R.Br., *Vinca major* L.; *Colocasia antiquorum* Schott.; *Calathea juncea* (L.) Benth. and Hook. F.; *Aster filifolius* Auct. non Vent.; *Cotyledon decussata* Sims.; *Cucumis nandinianus* Sond.; *Equisetum ramossissimum* Desf.; *Euphorbia helioscopia* L.; *Euphorbia mauritanica* L.; *Crotalaria nubica* Benth.; *Lessertia physodes* E. and Z.; *Albuca fastigiata* Dryand.; *Albuca altissima* Dryand.; *Bulbine narcissaeifolia* Salm-Dyck; *Dipcadi glaucum* Bkr.; *Dipcadi* sp. (N.H. No. 10061); *Scilla lanceaeifolia* Bkr.; *Urginea altissima* Bkr.; *Nemesia capensis* O. Kuntze; *Cestrum laerigatum* Schltr.; *Nicotiana glauca* R. Grah.; *Solanum incanum* Linn.; *Solanum nigrum* L.; *Solanum panduraciforme* E. Mey.; *Solanum rigescens* Jacq.; *Gnidia Burchellii* Meisn.

The results of the experiments with *Equisetum ramossissimum* appear to confirm the reports of farmers and natives that this plant when eaten by stock causes "drunkenness" and "shivering". The experimental sheep, when driven, fired much more rapidly than the

controls, but no shivering or "drunkenness" was noticed. Guinea-pigs injected subcutaneously with absolute alcohol extracts of the plant developed convulsions of the whole body. These convulsions could be brought on at any time by exciting the injected animals, for example, by beating on the cage.

Unfortunately a limited supply of the plant was available, with the result that only a few preliminary tests in regard to the solubility of the active principle could be conducted.

Nemisia capensis, which is held by farmers in Uniondale District to be extremely poisonous, especially when in the flowering stage, failed to produce any symptoms of poisoning in sheep drenched with a large amount of the fresh plant in the flowering stage and grown at Onderstepoort. It is possible that climatic conditions might affect the degree of toxicity of the plant. It may, however, be mentioned that comparatively large amounts of this plant in the dried state and flowering stage have been drenched to sheep without any ill-effects.

Nicotiana glauca known to be poisonous to ostriches has been proved toxic to rabbits and sheep producing symptoms typical of those of nicotine-poisoning.

Large amounts of the ripe fruit of *Solanum incanum* have been drenched to sheep, goats and rabbits without any deleterious effects, while relatively small quantities of the unripe fruit caused death in rabbits.

The ripe berries of *Solanum nigrum* are being used as a substitute for raisins and currants in puddings and are also freely eaten in the raw state especially by children. No untoward results have as yet been reported. The unripe berries proved to be very toxic to rabbits.

Fair amounts of the unripe and ripe fruit of *Solanum rigescens* proved to be non-toxic to rabbits.

The following plants, of which no toxic records [except *Urginea altissima* Bkr., which is mentioned by Bernhard-Smith (1923) to contain the same toxic principles as *Urginea scilla*] could be found in the available literature, were definitely proved toxic: *Dipcadi glaucum* Bkr.; *Gnidia Burchellii* Meisn.; *Solanum panduraciforme* E. Mey.; and *Urginea altissima* Bkr.

SUMMARY.

(a) Twenty-seven different species of plants were tested biologically.

(b) The symptoms produced in sheep and guinea-pigs appear to corroborate the evidence supplied by farmers as to the toxicity of *Equisetum ramosissimum* to stock.

(c) *Nicotiana glauca* R. Grah. (wild tobacco) has been proved toxic to sheep and rabbits.

(d) Small quantities of the green berries of *Solanum nigrum* L. caused death in rabbits, whereas the ripe berries both in the raw and cooked state have been and are still being extensively consumed by human beings without any ill-effects.

(e) The unripe and ripe berries of *Solanum rigescens* Jacq. produced no symptoms of poisoning in rabbits.

(f) *Urginea altissima* Bkr. has been proved toxic to sheep.

(g) The following plants have been proved toxic for the first time:

(i) *Dipcadi glaucum* Bkr.

(ii) *Gnidia Burchellii* Meisn.

(iii) *Solanum panduracforme* E. Mey.

LITERATURE.

- BERNHARD-SMITH, A. (1923). Poisonous Plants of all Countries. 2nd Edition, Boillière, Tindall & Cox, London.
- CURSON, H. H. (1928). Some Little-known South African Poisonous Plants and their Effects on Stock. 13th and 14th Repts. of D.V.E. & R., U. of S. Afr., pp. 203-230.
- EPSTEIN, D. (1930). Insulin and Insulin-like Substances. *Jnl. of Med. Assoc. of South Africa*, Vol. IV, pp. 722-724.
- EPSTEIN, D. (1926). The Action of Vinca on the Blood Sugar of Rabbits. *S.A. Med. Rec.*, Vol. 24, pp. 35-38.
- HUTCHEON, D. (1903). The Poisoning of Stock. *Agric. Jnl. of C. of G. Hope*, Vol. 23, p. 397.
- LANDER, G. D. (1926). Veterinary Toxicology. Boillière, Tindall & Cox, London.
- LONG, H. C. (1917). Plants Poisonous to Live Stock. Cambridge University Press.
- MARLOTH, R. (1917). The Common Names of Plants. The Specialty Press of South Africa, Ltd., Capetown.
- PAMMEL, L. H. (1911). A Manual of Poisonous Plants. The Torch Press, Cedar Rapids, Iowa.
- PHILLIPS, E. P. (1926). A Preliminary List of the Known Poisonous Plants found in South Africa. *Memoir No. 9*. The Government Printing and Stationery Office, Pretoria.
- THOMSON, R. B., and SIFTON, H. B. (1922). Poisonous Plants and Weed Seeds. University of Toronto Press.
- WILLIS, J. C. (1919). A Manual of Flowering Plants and Ferns. Cambridge University Press.

***Chrysocoma tenuifolia* Berg Poisoning in Angora Goats and the Development of Tolerance.**

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INTRODUCTION.

In the course of experiments conducted at Onderstepoort with *Chrysocoma tenuifolia* (bitterbossie)* it was noticed that certain quantities of the "bitterbossie" caused profuse diarrhoea and death in Angora goats, whereas, those animals which had been accustomed to the plant by administering small, that is, non-toxic, doses at the beginning of the experiments, tolerated toxic and lethal doses of the plant without any ill-effects. This was experienced in both the Willowmore and Colesberg varieties of the "bitterbossie."

* Investigations into the Cause of Alopecia (Kaalsiekte) in Kids and Lambs.
17th Report. Dir. Vet. Serv. Union of South Africa, 1931.

Investigations into the development of a tolerance to the "bitterbossie" were conducted in the course of the Alopecia experiments and subsequently these experiments were repeated with identical results. The results of these experiments are recorded below.

Mature Angora goats were utilized in these experiments, and it was proved that non-pregnant and highly pregnant goats were equally susceptible to the plant.

Drenching experiments to determine whether the plant decreased in toxicity during the process of drying and storage proved this not to be the case. After the finely ground plant had been spread out in the sun for nineteen days, it was found not to have lost any of its toxicity.

The diarrhoea in some of the animals engaged in these experiments was treated, either with a mixture of raw linseed oil (100 c.c.) and limewater (100 c.c.) or a mixture of raw linseed oil (100 c.c.), limewater (100 c.c.) and tannic acid (1 gram). The latter mixture was found to be superior to the former.

As the animals refused to take the plant voluntarily, they were drenched with the dry plant in the flowering stage by means of a stomach tube.

WILLOWMORE BITTERBOSSIE.

(a) TOXICITY TESTS.

Each of the goats 29201 and 29217 received 2,000 grams of the plant in the course of three consecutive days. Goat 29201 developed a profuse diarrhoea within forty-eight hours after commencement of drenching, when the animal had received 2,000 grams of the plant. It was not treated and died on the twenty-fourth day of the experiment. The incessant diarrhoea caused a pronounced state of cachexia and weakness. Goat 29217 exhibited a profuse diarrhoea within thirty-six hours after the first dose, and died within a few hours after it had received the third dose. Details of the experiments will be found in the accompanying appendix.

I might mention that the toxicity of the "bitterbossie" was repeatedly verified in the course of the Alopecia experiments.

(b) TOLERANCE TESTS.

As the plant in the above amounts caused such harmful effects and death in goats, it was decided to commence drenching with smaller doses and to increase these after a time, as follows:—

Goat 29203 received daily* as a preliminary treatment, 200 grams of the plant from 10.11.30 to 30.11.30, that is, a total of 3,600 grams in three weeks. The tolerance of the animal to the "bitterbossie" was then tested by giving it 8,400 grams of the plant in the course of fourteen days. No diarrhoea or any other symptom of ill-health was developed.

* Daily implies week-days only.

Goat 29205 received a total of 2,000 grams of the plant from 10.11.30 to 20.11.30 at the rate of 200 grams daily. The tolerance test consisted of a dose of 10,800 grams of the plant given in the course of three weeks.

In spite of the fact that no symptoms of ill-health developed, the animal was found dead on the morning of the twenty-second day of the tolerance test. Heartwater was suspected.

COLESBERG BITTERBOSSIE.

(a) TOXICITY TESTS.

The undermentioned experiments will suffice to prove that the Colesberg "bitterbossie" was more poisonous than the Willowmore variety. Both varieties utilized in these experiments were in the early flowering stage and in a dry state.

Each of *goats* 29219 and 29210 received 400 grams of the Colesberg "bitterbossie" on each of two consecutive days. Both animals developed a pronounced diarrhoea within thirty hours after the first dose the former dying within forty hours and the latter within sixty hours after the first dose.

Each of *goats* 18787, 29216 and 29218 received 200 grams of the plant on each of two consecutive days. Goats 18787 and 29218 exhibited profuse diarrhoea within forty-eight hours after the first dose, whereas in goat 29216 diarrhoea appeared within thirty-six hours. Goats 18787 and 29216 recovered after treatment, whereas 29218, which was not treated, died within seventy-two hours after the first dose.

Goat 29202 received 100 grams of the plant on each of three consecutive days. Within twenty-four hours after the last dose pronounced diarrhoea developed, death occurring on the sixth day of the experiment.

(b) TOLERANCE TESTS.

Goat 29211.—From the accompanying appendix it is evident that 200 grams of the plant given on each of two consecutive days produced pronounced diarrhoea, which disappeared within three days after treatment. The animal then received 50 grams of the plant daily for a period of twenty-six days ending on 5.12.30. Its resistance was then tested by administering the plant in the following quantities: 6.12.30, 300 grams; 8.12.30, 300 grams; 9.12.30, 400 grams; 10.12.30, 400 grams. On 11.12.30 profuse diarrhoea appeared, but recovery took place on the third day of treatment.

On 15.12.30 another dose of 300 grams caused slight diarrhoea which disappeared spontaneously. In the course of the following twenty days this animal received 4,000 grams of the plant without any ill-effects.

Goat 29212 developed diarrhoea after having received a dose of 200 grams of the plant on each of two consecutive days. Recovery soon set in after treatment. Subsequently it received 50 grams of the plant daily for three weeks, and then 150 grams on each of two consecutive days.

In the course of the following eight days a daily dose of 300 grams was given and diarrhoea set in on the ninth day, but disappeared within three days after treatment. After this animal had received a preliminary treatment of 50 grams of the "bitterbossie" daily for three weeks and 150 grams on each of two consecutive days it required 2,100 grams of "bitterbossie" to produce diarrhoea whereas before treatment 400 grams of this plant sufficed to produce this effect.

Goat 29206.—200 grams of the plant given on each of two consecutive days produced profuse diarrhoea, which disappeared within two days after treatment. In the course of the following six days this animal received a daily dose of 50 grams of the plant, and on each of the following two days 100 grams. A slight diarrhoea then appeared, and became very pronounced when another dose of 100 grams of the plant was given. Treatment, however, caused a speedy recovery. The animal was then able to tolerate 100 grams of the plant given on each of four consecutive days without any deleterious effects.

DISCUSSION.

Willowmore Bitterbossie.—2,000 grams of this plant in the dry state given within a period of three days to susceptible goats (29201 and 29217) caused diarrhoea and death, whereas 3,600 grams over a period of three weeks produced no ill-effects in goat 29203*. In order to produce tolerance two goats (29203 and 29205) received 200 grams of the plant daily for twenty-one days. The tolerance of goat 29203 was then tested by administering a total quantity of 8,400 grams of the plant over a period of fifteen days, that is, an average daily dose of 560 grams. From the above it is evident that 2,000 grams of the plant given on three consecutive days caused death in untreated goats, whereas 8,400 grams administered in the course of fifteen days produced no ill-effects in an animal which had received non-toxic amounts (200 grams) of the plant for a period of twenty-one days.

After goat 29205 had received a total quantity of 2,000 grams of the plant at the rate of 200 grams daily, as a preliminary treatment, it was given 10,800 grams of the plant over a period of three weeks, without developing any symptoms of "bitterbossie" poisoning.

Goats 29203 and 29205, in comparison with 29201 and 29217, therefore, showing a striking tolerance to the "bitterbossie" after having received non-toxic amounts of the plant over long periods.

Colesberg "Bitterbossie."—Six goats were employed in the tests to ascertain the toxic doses of this plant. 300 grams of the plant given over a period of three days caused death in goat 29202, and in goats 29219 and 29210 a quantity of 800 grams given within two days produced death. Goats 18787 and 29216 developed pronounced symptoms of poisoning after having received 400 grams of the plant within two days, but recovered after treatment, whereas goat 29218, which had received the same quantity of plant but had not been treated, died within seventy-two hours.

* It appears that the Willowmore "bitterbossie" produces no symptoms of poisoning when given at the rate of 200 grams daily to "untreated" goats, whereas average amounts of 600 grams daily caused pronounced diarrhoea and death after a total amount of 2,000 grams had been administered.

In the tolerance test three goats (29211, 29212, and 29206) were used. In order to exclude the possibility of a difference in susceptibility being interpreted as tolerance to the plant, these three animals received the same doses of the plants as goats 18787, 29216 and 29218, namely, 400 grams within two days, with the result that they developed profuse diarrhoea, which disappeared after treatment. It is, therefore, clear that the three goats utilized in the tolerance test showed a susceptibility equal to the other goats used in the Colesberg "bitterbossie" experiment.

After goat 29211 had received the preliminary treatment of 50 grams of the plant daily for a period of twenty-six days, it required 1,400 grams at an average daily dose of 350 grams to produce the characteristic diarrhoea, whereas 400 grams given within two days sufficed to produce diarrhoea before the animals had received non-toxic amounts of the plant over a prolonged period. From the appendix it will be seen that during the last twenty days of the experiment this animal received a total of 4,000 grams of the plant without any ill-effects.

Goat 29212 received 50 grams of the plant daily for a period of three weeks and then 150 grams on each of two consecutive days. Its tolerance to the plant was then tested by administering 300 grams daily. Diarrhoea appeared on the ninth day, that is, after the animal had received a total quantity of 2,400 grams. From the above it is apparent that before treatment with small quantities of the plant 400 grams given within two days sufficed to produce a profuse diarrhoea, whereas after treatment 2,400 grams were required. This is ample proof of the tolerance to the plant developed by this animal in the course of the treatment with non-toxic amounts of the plant.

Goat 29206 received 50 grams of the plant daily for a period of six days only. From subsequent tolerance tests it appeared that this period did not suffice for the production of a high degree of tolerance as 200 grams of the plant given at the rate of 100 grams daily again produced diarrhoea. Treatment resulted in a speedy recovery. The animal was now able to tolerate a dose of 400 grams of the plant given at the rate of 100 grams daily without any deleterious effects.

CONCLUSIONS.

From the foregoing experiments it is evident that Angora goats, after having undergone a preliminary treatment with non-toxic doses of the "bitterbossie," can tolerate to a remarkable degree such quantities of the plant as would have caused death in untreated animals.

The degree of tolerance appears to depend on the length of the period of preliminary treatment with non-toxic doses.

APPENDIX.

Goat No.	Nature of Test.	Origin of Plant.	Details of Dosage.	Remarks.
29201	Toxicity Test....	Willowmore.....	8.1.31, 800 grams; 9.1.31, 400 grams; 10.1.31, 800 grams	Diarrhoea within 48 hours after first dose and death on 1.2.31.
29217			5.1.31, 800 grams; 6.1.21, 400 grams; 7.1.31, 800 grams	Diarrhoea within 36 hours after first dose and death on 7.1.31. Remained healthy.
29203	Tolerance Test....		10.11.30-30.11.30, 200 grams daily; 1.12.30-15.12.30, 400 grams daily; on each of 1.12.30, 3.12.30, 5.12.30, 7.12.30, 9.12.30, 11.12.30, 13.12.30, and 15.12.30, 400 grams, additionally i.e. 800 grams daily on these dates	This animal developed no untoward symptoms up to 11.12.30, and was found dead the next morning. Heartwater was suspected.
29205			10.11.30-20.11.30, 200 grams daily; 21.11.30-11.12.30, 400 grams daily; on each of 24.10.30, 26.11.30, 28.11.30, 1.12.30, 3.12.30, 5.12.30, 7.12.30, 9.12.30, and 11.12.30, an additional 400 grams i.e., 800 grams daily on these dates	
29219	Toxicity Test....	Colesberg.....	3.11.30, 400 grams; 4.11.30, 400 grams.....	Pronounced diarrhoea within 30 hours and death within 40 hours after the first dose.
29210			3.11.30, 400 grams; 4.11.30, 400 grams.....	Pronounced diarrhoea within 30 hours and death within 60 hours after the first dose.
18787			25.2.31, 200 grams; 26.1.31, 200 grams.....	Profuse diarrhoea within 48 hours after the first dose; recovered after treatment.
29216			5.1.31, 200 grams; 6.1.31, 200 grams.....	Profuse diarrhoea within 36 hours after first dose; recovered after treatment.
29218			6.11.30, 200 grams; 7.11.30, 200 grams.....	Pronounced diarrhoea within 48 hours and death within 72 hours after first dose.
29202			5.2.31, 100 grams; 6.2.31, 100 grams; 7.2.31, 100 grams	Profuse diarrhoea within 80 hours and death within six days after first dose.
29211	Tolerance Test....		6.11.30, 200 grams; 7.11.30, 200 grams; 11.11.30-5.12.30, 50 grams daily; 6.12.30, 300 grams; 8.12.30, 300 grams; 9.12.30, 400 grams; 10.12.30, 400 grams; 15.12.30, 300 grams; 18.12.30, 200 grams; 19.12.30, 200 grams; 20.12.30, 300 grams; 22.12.30-24.12.30, 300 grams daily; 27.12.30, 300 grams; 29.12.30-31.12.30, 300 grams daily; 2.1.31-5.1.31, 200 grams daily	8.11.30, pronounced diarrhoea; recovered after treatment. 11.12.30, pronounced diarrhoea; recovered after treatment. 17.12.30, slight diarrhoea; recovered spontaneously. From 18.12.30, animal developed no symptoms of ill-health.
29212			6.11.30, 200 grams; 7.11.30, 200 grams; 11.11.30-30.11.30, 50 grams daily; 1.12.30, 150 grams; 2.12.30, 150 grams; 3.12.30-10.12.30, 300 grams daily	8.11.30, profuse diarrhoea; recovered after treatment. 11.12.30, profuse diarrhoea; recovered after treatment.
-			6.11.30, 200 grams; 7.11.30, 200 grams; 11.11.30-16.11.30, 50 grams; 17.11.30, 100 grams; 18.11.30, 100 grams; 19.11.30, 100 grams; 22.11.30-25.11.30, 100 grams daily	8.11.30, profuse diarrhoea, recovered after treatment. 19.11.30, slight diarrhoea. 20.11.30, slight diarrhoea. From 22.11.30, animal developed no symptoms of ill-health.
29206				

A Study of the Factors concerned in the Determination of the Toxicity of *Cotyledon orbiculata* L.*

By D. G. STEYN, B.Sc., Dr.Med.Vet., Veterinary Research Officer,
Onderstepoort.

This subject will be treated under the following headings:—

- I. Introduction.
- II. Literature.
- III. Procedure and Technique.
 - (a) Plant and Soil.
 - (b) Contents of the Pot.
 - (c) Watering of the Plants.
 - (d) Preliminary Experiments.
 - (e) Preparation of the Extracts for Injection.
- IV. Observations made in the course of the Experiments.
 - (a) The Plants.
 - (b) The Extracts.
- V. Discussion.
 - (a) Literature.
 - (b) Onderstepoort Experiments.
 - (c) Effect of Sunlight.
 - (d) Effect of Fertilizers.
 - (e) Physical Properties of the Leaves of the different Plant Specimens.
 - (f) Toxicity of the different Specimens of *Cotyledon orbiculata*.
- VI. Conclusions.

* Mr. C. A. Smith, who recently returned from Kew, identified this plant as *Cotyledon leucophylla* Smith.

INTRODUCTION.

It is common knowledge that one and the same medicinal plant growing in different countries varies in its active principle content to a considerable extent. As an example *Papaver somniferum* may be quoted. Furthermore, the toxicity of *Solanum nigrum* varies to such an extent that it is regarded as harmless in one country and poisonous in another.

The same holds good in the case of poisonous plants growing in different countries and even in different localities in the same country. It will be of interest to note that in the case of *Cotyledon orbiculata* specimens used in the undermentioned experiments it was found that there was a striking difference in the toxicity of the individual plants collected from the same locality at the same time and at the same stage of development.

In the course of investigations into the toxicity of plants the author has made the following observations:—

- (a) Specimens of *Cotyledon ventricosa* and *Cotyledon walliehii* sent in from different parts of the Union varied considerably in toxicity in spite of the fact that they were at the same stage of development.
- (b) The mature drupes collected from one and the same syringa tree (*Melia azedarach*) growing at Onderstepoort were found to vary in toxicity in different years. Also the mature drupes forwarded to Onderstepoort from different parts of the Union varied in toxicity.
- (c) A specimen of *Cotyledon orbiculata* (Steyn, 1929), which was brought from the Magaliesberg, was broken into two parts, one of which was planted in sandy soil and the other in heavy clay soil at Onderstepoort. Five and a half months after transplantation the sandy soil specimen had lost half of its toxicity, whereas the heavy clay soil one had doubled its toxicity.
- (d) *Geigeria zeyheri* (Steyn, 1929) growing at Pretoria North was found to be toxic to sheep during December, 1927, and January, 1928, whereas from February to September, 1928, three times the lethal dose had no deleterious effects on sheep. In October, 1928, however, the lethal dose was the same as in December, 1927.
- (e) 50 grams of a *Psilocaulon* sp. (Nat. Herb. No. 8819) collected by the author on the farm Tweefontein, Willowmore district, during a severe drought killed rabbits within one hour. Specimens of this plant were planted on the rockery in the Onderstepoort poison garden. After an interval of two months the plant was growing luxuriantly and quantities up to 120 grams failed to produce any symptoms of poisoning in rabbits.
- (f) Specimens of *Cotyledon ventricosa* and *Cotyledon walliehii* were brought from the Willowmore district and planted on the rockery in the poisonous plant garden at Onderstepoort.

In their natural state these two *Cotyledon* spp. are found growing under shrubs and bushes apparently in order to avoid the effects of direct sunlight. At Onderstepoort they were exposed to the direct sunlight with the result that all the leaves dropped off within fourteen days of planting. In the course of the following three months the thick stems, which remained green and fresh, produced a few stunted rudimentary leaves which showed no tendency to increase in size. As no change in the growth had taken place within four months of transplantation it was decided to shade the plants. The result was that numerous normal leaves appeared within ten days and grew to maturity within two months. In addition some *Cotyledon ventricosa* specimens, which had been planted out in a bed and which, up to four months after transplantation showed no tendency to produce any leaves or flowers, were removed and planted on a shaded spot on the rockery. Within a week leaves, which matured within three months, appeared.

The above observations prompted the author to conduct the experiments to be described in this article.

LITERATURE.

Determinations of the chief alkaloidal active principles of medicinal plants for four successive years, from 1907 to 1910, grown in the same locality, show that these have undergone a marked deterioration in potency, especially in years of low temperatures and deficient sunshine (Burmah, 1911). Ransom and Henderson (1912) investigated the effect of cultivation and fertilization on the growth of *Atropa belladonna* and on the alkaloidal content of its leaves. Plots were used in which the seeds were sown and various fertilizers applied. They found no appreciable difference in the alkaloidal content, although it appears to be increased by full exposure to sunlight.

Long (1917) in briefly discussing the effects of soil, climate and cultivation on the toxic properties of plants, states that in many cases plants vary considerably in toxicity according to soil, light, moisture, etc. This variation in toxicity is very striking in the case of *Solanum nigrum*, which in some countries is looked upon as harmless, while in others it is considered poisonous.

In the course of experiments conducted with *Atropa belladonna* at the Arlington Experimental Farm, Virginia, (Long, 1917), it was found that in twenty-four plants the alkaloidal content varied from 0.334 to 0.700 per cent. in 1910 and in 1911 it varied in fifty-nine plants from 0.306 to 0.766 per cent., this being the average of five pickings. In the individual plants alkaloidal content varied from 0.200 to 0.925 per cent.

The above variations in the alkaloidal content occurred in plants in the same stage of development and growing in the same locality under identical climatic and soil conditions.

Long, furthermore, publishes in tabular form the results of experiments conducted at the Wellcome Materia Medica Farm, Kent, to determine the effects of farmyard manure, nitrate, calcium cyanamide, basic slag, superphosphate and potash on the alkaloidal content of

Atropa belladonna. The results were variable and inconclusive. It is, however, of interest to note that very low figures were obtained in one year (1907). The reason for this is put down as "probably due to the seasonal conditions."

The following is an extract from the October monthly report of Marais (1930), extension officer, Maclear, Cape Province, in regard to the effect of fertilizers on the palatability of vetches: "I have investigated this matter very carefully and came to the following conclusions: (a) The palatability of vetches is in direct proportion to the fertility of the soil on which they are grown. The better the soil, the more greedily the vetches are eaten by stock.

(b) The humus and possibly also the phosphorus content of the soil influences the palatability in some or other way. In two cases where the animals decidedly refused to partake of the vetches they were grown on very poor soil without the addition of fertilizers. In addition the vetches developed no nodules. The humus-content of the soil was extremely low."

McCarrison (1924) studied the effects of nitrogen, phosphorus and potash, either alone or in combination, and cattle manure on the yield of millet. He writes: "The results shown in Tables I and II appear to indicate (1) that millet grown on soil manured continuously with cattle manure was of higher nutritive value for pigeons, than millet grown on soil manured with chemical manures, even when the chemical manure was a complete one; (2) that the nutritive value of the grain varied with the manure used in its cultivation; and (3) that the grain grown on an exhausted soil was of low nutritive value. Beyond these broad general indications it would be unsafe to go, but it is significant that while a complete chemical manure may greatly increase the yield of grain, its nutritive value falls far below that of the same grain grown on soil manured with natural manure."

Furthermore, he remarks as follows: "It will be noted from Table III that in Group IX the millet grown on soil which received cattle manure markedly delayed the onset of polyneuritis, and that the average period before its onset was longer in this group than in any other. This result, taken in conjunction with the first experiment, which also indicated a higher vitamin B value for this sample, would seem to justify the conclusion that the grain grown on soil which received natural manure was of higher vitamin B value than that grown on soil which received artificial manure."

The same author (1926) conducted experiments in order to determine the effect of manurial conditions on the nutritive and vitamin values of millet and wheat and the following paragraphs in his summary are of great interest:—

"(2) The soil manured with natural or farm-yard manure yielded a millet or wheat of higher nutritive value than the same soil when manured with a complete manure or so-called 'artificial' manure. Soil that had not been manured at all for many years, but which had been continuously under crops, yielded a millet of very low nutritive value, which was actually harmful to adult pigeons. It seems probable that different grains may be affected in different ways by want of manure . . .

(4) This difference appears to be due in considerable part to differences in the vitamin content of the grain: Wheat grown on soil treated with cattle manure contained more vitamin A than wheat grown on soil treated with complete chemical manure; millet grown on soil treated with cattle manure contained more vitamin B than millet grown on soil treated with complete chemical manure."

Khalil studied the effect of drying on the micro-biological processes in soils and recorded some interesting facts in his summary:—

"(1) Drying considerably reduced the bacterial content of the soil, but, on subsequent moistening and incubation, dried soil showed higher numbers than permanently moist soils. (2) Air-drying caused a rapid conversion of ammoniacal to nitrate nitrogen. (3) Drying increased the nitrogen fixing capacity of the soil (solution tests) and its ammonifying and nitrifying power (soil tests). (4) No evidence could be obtained in support of the view that the microflora of dried soils is more efficient than that of moist soils:—

- (a) In ammonification and nitrification solution tests, when approximately equal numbers of bacteria were introduced, there was no difference in the ammonifying and nitrifying capacity of dried and moist soils.
- (b) Inoculation of dried soil with a filtrate from moist soil did not reduce its ammonifying and nitrifying power (soil tests).
- (c) The drying of partially sterilized soil (subsequently re-inoculated) increased its ammonifying and nitrifying capacity (soil tests). Drying renders the soil organic matter more easily decomposable: (a) The organic matter in drier soil was more rapidly ammonified and nitrified than that in moist soil. (b) Soils to which organic matter had been added before drying showed greater increase in microbiological activity as a result of drying than soils which had received no addition.

(6) For normal soils, soil extract agar and glycerine nitrate agar gave higher bacterial numbers than Thornton's medium. The latter gave the highest results for soils incubated after treatment with hydrocyanic acid gas."

Ghosh and Krishna (1930) found the alkaloidal content of *Ephedra* spp. growing in areas with a high rainfall lower than that of those spp. growing in drier areas.

In addition they established that the alkaloidal content of the *Ephedra* spp. is at its lowest during the rainy season.

The following quotation from Texas Bulletin 422 (Fraps, 1931) is of interest: "Varieties of corn varied little in composition, including protein, but the protein content varied considerably according to the locality in which the corn was grown. The correlation between rainfall, January through July, and protein content was $-576 \pm .072$. Slightly less lime and phosphoric acid were in the corn grown in two localities than in the samples grown at the other five places."

PROCEDURE AND TECHNIQUE.

In the course of this description it will be evident that extreme care had been exercised in order to prevent as far as possible errors that may influence the determination of the M.L.D.

(a) PLANT AND SOIL.

The plant specimens utilized in these experiments were collected on 14.4.30 from the top of the Magaliesberg near the small "Wonderboom". These specimens were mature plants in the post-seeding stage and still showed the remains of the flower stalks of the previous season.

Twelve of the largest sized plants obtainable were dug up and immediately planted in twelve iron-pots (see photographs) prepared for this purpose. The plants were carefully dug up so as not to damage the roots and all the soil removed from the roots in order to render the conditions of adaptability identical. Each pot (with soil) weighed 38 Kg. before the specimens were planted in them.

The sandy soil was obtained from the new poisonous plant garden and the black clay soil from the old poisonous plant garden. The Calcium and Phosphorus content of the same species of dried grass collected on the spots from which the soil was collected was determined * and yielded the following results:—

Grass from the black clay soil	0.247% P_2O_5 and 0.449% CaO
Grass from the sandy soil	0.154% P_2O_5 and 0.273% CaO.

(b) CONTENTS OF THE POTS.

It was fully realized that this experiment was a "shot in the dark" and the intention was to utilize the results obtained as a basis for further experiments in this connection.

Pots Nos. I and IV contained heavy black clay soil.

Pots Nos. II and V contained heavy black clay soil + 40 grams of sodium phosphate.

Pots Nos. III and VI contained heavy black clay soil + 200 grams of dry sheep manure.

Pots Nos. VII and X contained sandy soil.

Pots Nos. VIII and XI contained sandy soil + 40 grams sodium phosphate.

Pots Nos. IX and XII contained sandy soil + 200 grams of dry sheep manure.

Pots Nos. IV, V, VI, X, XI and XII were kept in diffused light on the verandah of the chemical block and Pots Nos. I, II, III, VII, VIII and IX in the open air and thus exposed to the direct sunlight.

* I am indebted to Mr. J. S. Otto, M.Sc., of the Department of Chemistry, for these determinations.

From the above, it is obvious that the effects, if any, on the toxicity of *Cotyledon orbiculata* of (a) diffused and direct sunlight, and (b) different soils and fertilizers, namely sodium phosphate and sheep manure were to be tested.

(c) WATERING OF THE PLANTS.

All the plants were watered at the same time and those kept in the open air were covered by a tarpaulin in order to prevent them from getting any additional moisture.

It must be mentioned that it is impossible to control accurately the water supply of the plants by the weights of the pots as leaves dropped off as a result of being attacked by insects or from other causes and a large number of leaves were used for the tests. In addition a large number of young leaves appeared. It was, however, attempted to keep the water supply as uniform as possible.

(d) PRELIMINARY EXPERIMENTS.

Preliminary experiments were conducted in order to determine (1) whether during the process of extraction the degree of agitation has any effect on the amount of poison extracted by the solvent; (2) whether the comminution of the *Cotyledon* leaves has any influence on the degree of extractability of the poison; (3) whether there is any difference in the toxicity of leaves in the different stages of development; (4) the average toxicity of mature *Cotyledon orbiculata* plants growing on the Magaliesberg; (5) the relative toxicity of the fluid and solid parts of the minced leaves; and (6) whether the leaves of the same plants are equally toxic.

The above points were determined by the same method as that employed in the main experiment, namely, by injecting subcutaneously into guinea-pigs evaporated 96 per cent. alcoholic extracts of the leaves, as no method of detecting and estimating by chemical means the active principle of this plant is known.

It was necessary to determine these points in order to evolve a standard method of extraction.

EXPERIMENT I.

To determine whether the degree of agitation has any effect on the amount of poison extracted by the solvent. The following procedure was adopted:

- A. The minced leaves were shaken up with an equal weight of 96 per cent. alcohol until a thorough suspension was formed. This suspension was then left standing for twenty-four hours.

Result.—The M.L.D. per 500 gram guinea-pig was found to be approximately 15 grams of the fresh leaf.

- B. A suspension of the leaves was prepared as above and shaken at two-hourly intervals for the first ten hours of extraction and then left standing for a further fourteen hours.

Result.—The M.L.D. per 500 gram guinea-pig was found to be approximately 8 grams of the fresh leaves.

C. A suspension of the leaves prepared as above was continuously shaken by machine for first eight hours of extraction and then left standing for a further sixteen hours.

Result.—The M.L.D. per 500 gram guinea-pig was found to be approximately 8 grams of the fresh leaves.

D. A suspension of the leaves prepared as above was continuously shaken by machine for twenty-four hours.

Result.—The M.L.D. per 500 gram guinea-pig was found to be 8 grams of the fresh leaves.

Conclusions.

The extractability of the *cotyledon* poison depends to a certain extent on the degree of agitation of the suspension. It is, however, not necessary to shake the suspension continuously in order to saturate the solvent with the poison. The fact that the least poison is extracted by the solvent when the suspension is only mixed and not shaken subsequently is due to the settling of the solid part of the suspension at the bottom of the flask, thus preventing the solvent from being in constant contact with solid parts of the mixture.

EXPERIMENT II.

To ascertain whether the degree of division of *Cotyledon* leaves has any influence on the degree of extractability of the poison.

A. Leaves cut into 5×5 cm. pieces and immersed in an equal weight of 96 per cent. alcohol for twenty-four hours.

Volume of filtrate before evaporation = 200 c.c.

Result.—The M.L.D. per 500 gram guinea-pig was found to be approximately 40 grams of the fresh leaves.

B. Leaves cut into 2.5×2.5 cm. pieces and extracted as in A.

Volume of filtrate before evaporation = 236 c.c.

Result.—The M.L.D. per 500 gram guinea-pig was found to be approximately 35 grams of the fresh leaves.

C. Leaves cut into 1.25×1.25 cm. pieces and extracted as in A.

Volume of filtrate before evaporation = 236 c.c.

Result.—The M.L.D. per 500 gram guinea-pig was found to be approximately 22 grams of the fresh leaves.

D. Leaves minced by means of an ordinary mincing-machine and extracted as in A.

Volume of filtrate before evaporation = 290 c.c.

Result.—The M.L.D. per 500 gram guinea-pig was found to be approximately 15 grams of the fresh leaves.

E. Leaves minced very finely by means of a grinding mill and extracted as in A.

Volume of filtrate before evaporation = 300 c.c.

Result.—The M.L.D. per 500 gram guinea-pig was found to be approximately 12 grams of the fresh leaves.

All the above leaves were collected from the same plant, and the extracts shaken at two-hourly intervals for the first eight and last four hours of extraction.

Conclusions.

The degree of division of the leaves of *Cotyledon orbiculata* has a striking influence on the amount of poison extracted by the solvent. It will be noticed from the above experiments that the finer the state of division of the leaves the greater the volume of the filtrate. The increased toxicity of the extracts prepared from the leaves in the finest state of division is due to the liberation of a much larger quantity of fluid and the extraction of more toxin from the leaves than when these are in a coarser state of division.

EXPERIMENT III.

To determine whether there is any difference in the toxicity of leaves in the different stages of development.

In the past *Cotyledon orbiculata* has been the subject of many investigations conducted by the author and it has been repeatedly found that there is no difference in the toxicity of immature leaves (size $1 \times 2 \times 3$ cm.) and mature leaves ($2 \times 15 \times 23$ cm.) collected from the same plant at the same time. Despite these results the toxicity of young and old leaves of the same plant was again determined and found to be identical.

EXPERIMENT IV.

To determine the average toxicity of mature *Cotyledon orbiculata* plants growing on the Magaliesberg.

It was necessary to determine this point in order to calculate the amounts of plants to be utilized in the main experiment. In the various tests the M.L.D. of the fresh leaves of the different plants per 500 gram guinea-pig varied considerably, as is also evidenced by the tests with the plants grown in the pots and utilized in the main experiment.

As a result of these tests it was decided to utilize four guinea-pigs for each test and inject subcutaneously into them evaporated extracts equivalent to 5, 10, 25 and 65 grams of the fresh plant respectively.

EXPERIMENT V.

To determine the relative toxicity of the fluid and solid parts of the minced leaves.

In mincing *Cotyledon orbiculata* leaves it was noticed that some leaves minced into a soft porridge-like mass with no free fluid whereas others formed solid masses which could be easily separated from the clear watery juice. As the possibility existed that the poison might be fixed, to a certain extent at any rate, to the solid parts of the minced leaves, it was thought advisable to determine whether there was any difference in the toxicity of the fluid and solid parts of the minced leaves.

A weighed quantity of the minced leaves was placed in a tincture press and the fluid expressed. The fluid and solid parts were then weighed. The fluid was evaporated and injected subcutaneously into guinea-pigs. The solid part was extracted for twenty-four hours with an equal weight of 96 per cent. alcohol and the filtrate evaporated and injected subcutaneously into guinea-pigs. This determination was repeated three times with different leaves with the following results:—

1st Determination:

Solid part: M.L.D. per 500 gram guinea-pig=4 grams.
Fluid part: M.L.D. per 500 gram guinea-pig=4 grams.

2nd Determination:

Solid part: M.L.D. per 500 gram guinea-pig=4 grams.
Fluid part: M.L.D. per 500 gram guinea-pig=12 grams.

3rd Determination:

Solid part: M.L.D. per 500 gram guinea-pig=6 grams.
Fluid part: M.L.D. per 500 gram guinea-pig=10 grams.

In view of these varying results it was decided to mix the fluid and solid parts of these leaves in a mortar until a uniform paste was formed before weighing off the necessary amounts of minced leaves.

EXPERIMENT VI.

To determine whether the leaves of the same plant are equally toxic.

Result.—Three mature leaves collected from the same plant were utilized and found to be equally toxic.

(c) PREPARATION OF THE EXTRACTS FOR INJECTION.

As a result of Kamerman's (1926) work on various *Cotyledon* spp. the extracts were prepared with 96 per cent. alcohol. The tests were to be made at intervals of fourteen days throughout the course of fifteen months, but unfortunately this procedure could not be adhered to as after a time there were too few leaves to allow of a test every fourteen days and in addition some of the plants died. At each test 110 grams of the freshly minced leaves of each plant were extracted for twenty-four hours with an equal weight of 96 per cent. alcohol. This extract was filtered and the filtrate evaporated to 11 c.c., thus removing all traces of alcohol. During the process of filtration the contents of the flask containing the mixture of minced leaves and 96 per cent. alcohol were emptied into the filter so as to allow all the fluid to run off from the solid part of the extract. This evaporated extract was then injected subcutaneously into guinea-pigs in the following amounts: 0.5 c.c., 1.0 c.c., 2.5 c.c. and 6.5 c.c., equivalent to 5, 10, 25 and 65 grams of fresh leaves respectively. It was intended to utilize mature leaves of equal sizes only, but the fact that the leaves of those plants growing in diffused light soon began to wilt and drop off and the appearance of abnormally small leaves (see photographs) made this procedure impossible.

After the evaporation of the extracts to 11 c.c. the sides of the containers were washed off thoroughly in order to collect all the poison in the fluid to be injected. Before each injection the extract was well mixed by drawing it into the syringe and ejecting the contents with full force into the remainder of the extract in the container. It is of the utmost importance in determining the M.L.D. subcutaneously that the injections be made subcutaneously and not intramuscularly or intraperitoneally. In order to allow of the correct amount of extract to be injected each time the quantities to be injected were drawn into the syringe with the needle fixed to it. The needle should also be filled with the extract as otherwise the volume injected would be the volume shown by the syringe minus the volume of the needle. In the latter case the error is considerable when 0.5 c.c. of extract is to be injected.

In order to prevent any backflow of the injected extract a fairly long and thin needle was inserted as far as possible into the subcutaneous tissues before the injection was made and on extracting the needle the point of puncture was firmly compressed during, and rubbed between the fingers after, the removal of the needle. The syringe and needle were rinsed with 96 per cent. alcohol after the injection of each plant extract.

The M.L.D. was calculated per 500 gram guinea-pig and guinea-pigs of equal size were utilized as far as possible.

The same measuring cylinder, mincing machine and syringe were used throughout the experiment.

Eleven hundred and fifty-six guinea-pigs were utilized in these experiments. A large number of animals were employed in order to reduce individual idiosyncrasy and tolerance to a minimum.

OBSERVATIONS MADE IN THE COURSE OF EXPERIMENTS.

(a) THE PLANTS.

There was no appreciable difference in the growth of the specimens planted in the black clay soil (Pots Nos. I to VI) and those growing in the sandy soil (Pots Nos. VII to XII). The plants in the pots with fertilizers (namely sodium phosphate and sheep manure) showed much better growth than those specimens growing in the unfertilized pots (See Figs. V and VI).

The normal immature and mature leaves of the plants in the diffused light (Pots Nos. IV, V, VI, X, XI and XII) began to wilt and drop off from the end of the first month until none were left at the end of the fourth month after transplantation (see Figs. I, II, III and IV). In the meantime long, narrow and thin dark greenish leaves, which were much smaller in size than the normal leaves and did not show the characteristic white powder on their surfaces and the dark-red edges, appeared. These abnormal leaves, many of which dropped off when very young, had to be utilized for the tests from the end of the fourth month.

COMPARATIVE TABLE (EXTRACTED FROM

Pot No.	M.L.D. per 100 gm. Guinea- pig before Trans- plan- ta- tion.	Gm.	Gm.	M.L.D.—After Transplantation on Dates as given Below.											
				Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
I.	6	10	10	20	20	20	20	8	Not tested	8	12	12	12	12	4
II.	8	8	20	30	20	25	12	10	Not tested	25	12	12	12	6	6
III.	8	8	12	12	8	12	6	4	Not tested	6	6	6	6	6	6
IV.	20	55	65	70	65	65	65	65	Not tested	65	20	20	20	15	15
V.	8	12	8	15	10	30	12	20	Not tested	6	6	4	4	4	4
VI.	25	25	30	30	65	65	25	25	Not tested	30	65	15	15	15	6
VII.	8	12	12	6	8	8	8	8	Not tested	8	8	12	12	12	6
VIII.	8	12	8	6	12	12	6	6	Not tested	6	6	12	8	8	3
IX.	10	6	12	50	40	6	6	6	Not tested	12	6	15	8	8	6
X.	6	6	8	8	20	30	25	25	Not tested	15	15	8	8	12	6
XI.	6	6	6	6	12	20	20	30	Not tested	25	6	6	6	6	6
XII.	8	12	6	6	6	8	8	6	Not tested	6	3	3	3	3	3
Date of Test....	14.4.30	30.4.30	14.4.30	28.5.30	11.6.30	25.6.30	9.7.30	23.7.30	6.8.30	20.8.30	3.9.30	17.9.30	1.10.30	15.10.30	29.10.30
Date of Transplan- tation															

TABLES I TO XII). (SEE APPENDIX).

												Nature of Soil and Light.
Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	
Not tested	3	3	Not tested	25	Not tested	Not tested	Not tested	Not tested	12	Not tested	12	Grown on black clay soil and in direct sunlight.
Not tested	3	3	3	8	8	12	12	12	12	12	12	Grown on black clay soil + 40 gm. sodium phosphate and in direct sunlight.
6	3	3	3	6	6	30	8	8	8	8	8	Grown on black clay soil + 200 gm. sheep manure and in direct sunlight.
Not tested	3	Not tested plant dying	Plant dead	—	—	—	—	—	—	—	—	Grown on black clay soil and in diffused light.
Not tested	3	Plant dead	—	—	—	—	—	—	—	—	—	Grown on black clay soil + 40 gm. sodium phosphate and in diffused light.
Not tested	5	8	Plant dying	—	—	—	—	—	—	—	—	Grown on black clay soil + 400 gm. sheep manure and in diffused light.
Not tested	6	3	3	3	Not tested	Not tested	Not tested	Not tested	3	Not tested	Not tested	Grown on sandy soil and in direct sunlight.
6	3	3	3	5	5	12	8	5	3	Not tested	Not tested	Grown in sandy soil + 40 gm. sodium phosphate and in direct sunlight.
3	3	3	3	5	5	12	12	8	8	8	8	Grown on sandy soil + 200 gm. sheep manure and in direct sunlight.
Not tested	3	Plant dead	—	—	—	—	—	—	—	—	—	Grown on sandy soil and in diffused light.
6	Plant dead	—	—	—	—	—	—	—	—	—	—	Grown on sandy soil + 40 gm. sodium phosphate and in diffused light.
Not tested	Plant dying	Plant dead	—	—	—	—	—	—	—	—	—	Grown on sandy soil + 200 gm. sheep manure and in diffused light.
12.11.30	26.11.30	14.1.31	18.2.31	15.4.31	29.4.31	13.5.31	27.5.31	10.6.31	24.6.31	8.7.31	22.7.31	
14.4.30												

Furthermore, the plants growing in diffused light produced abnormally long flowerstalks, and the flowerheads, which were extremely small, wilted and dropped off before they reached maturity.

The six plants growing in diffused light commenced dying on 14.1.31 (Pot No. IV), 26.11.30 (Pot No. V), 14.1.31 (Pot No. VI), 26.11.30 (Pot No. X), 29.10.30 (Pot No. XI), and 27.11.30 (Pot No. XII) and were completely dead within six weeks with the exception of plant No. VI, which, when all the leaves had dropped off and all the stems, except one, were dead, was moved into the direct sunlight. Within a week a few normal leaves, that is, leaves of the normal shape and the typical dark-red edges and covered with a white powdery material, appeared on the live stem. These new leaves, however, did not mature but died within two months after their appearance.

(b) THE EXTRACTS.

On mincing the leaves of the various plants it was noticed that some of them formed a mass of porridge-like consistence with no or very little turbid fluid, whereas others separated into solid and liquid parts. The former on being mixed with the 96 per cent. alcohol formed a turbid milky mixture, while the latter remained clear and transparent. Intermediate stages were encountered. Very rarely it was noticed that the minced leaves of some plants formed a white flocculent precipitate on the addition of 96 per cent. alcohol.

Those extracts, which formed turbid mixtures with the 96 per cent. alcohol, filtered extremely slowly, whereas the clear ones did so with extreme rapidity. The filtrates of the various plant-extracts and of one and the same plant at the various intervals showed the following colours: dark-yellowish green, light-yellowish green, dirty dark-green and light-green. On evaporation some of these colours become more intense, whereas others turned dark pinkish. It was also evident that the leaves of some plants contained much more chlorophyll than others.

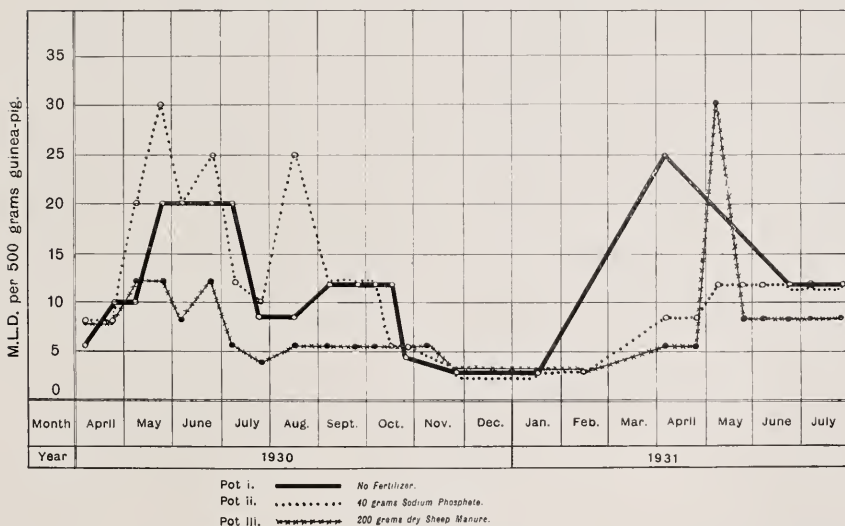
As the foregoing experiment was "a shot in the dark" and as further experiments to elucidate the factors concerned in the determination of the toxicity of plants are being in progress, it would at present not seem justifiable to rely too much on and to discuss too fully the results obtained by this preliminary experiment. The results of experiments which are now in progress will throw light on those obtained by this preliminary experiment and a full discussion on various points will then be possible.

Much more reliable information could have been obtained from this experiment, had not the six plants growing in the diffused light died. In addition quite a large number of leaves were necessary for the tests and this resulted in the six remaining plants growing in the direct sunlight having too few leaves to allow of further experimentation. These plants will be kept and as soon as a sufficient number of leaves are available, the tests will be continued.

The results tabulated in the comparative table can be best demonstrated by the following graphs.

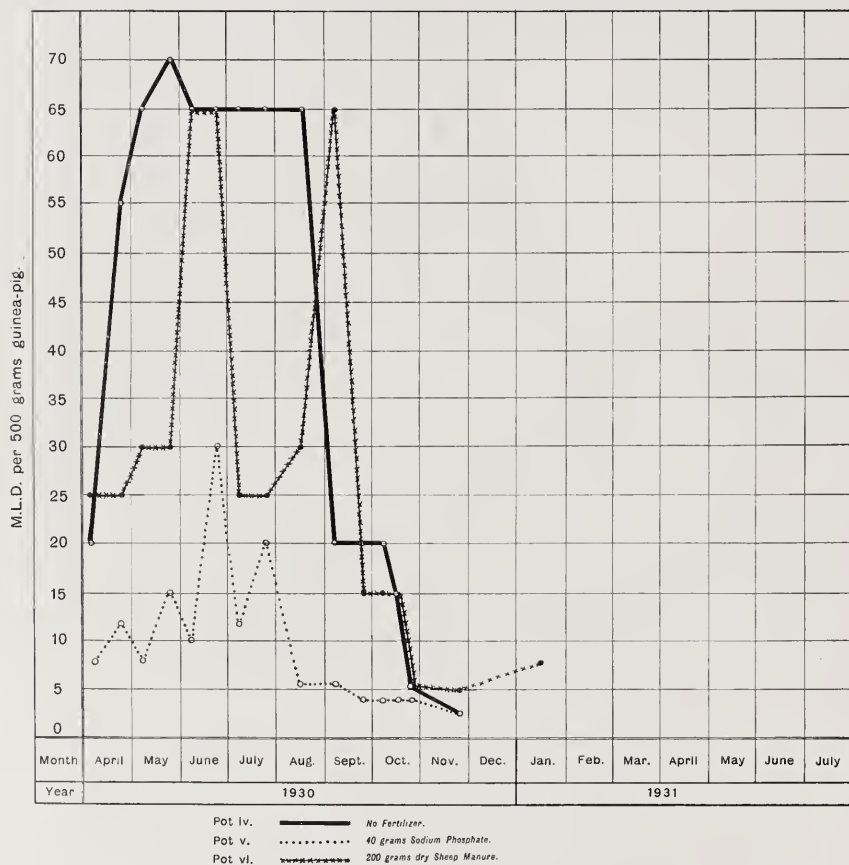
For the sake of simplicity the plants in the same group will be referred to as the unfertilized plant, the sodium phosphate plant and the sheep manure plant.

Graph I.—In Direct Sunlight (Black Clay Soil).



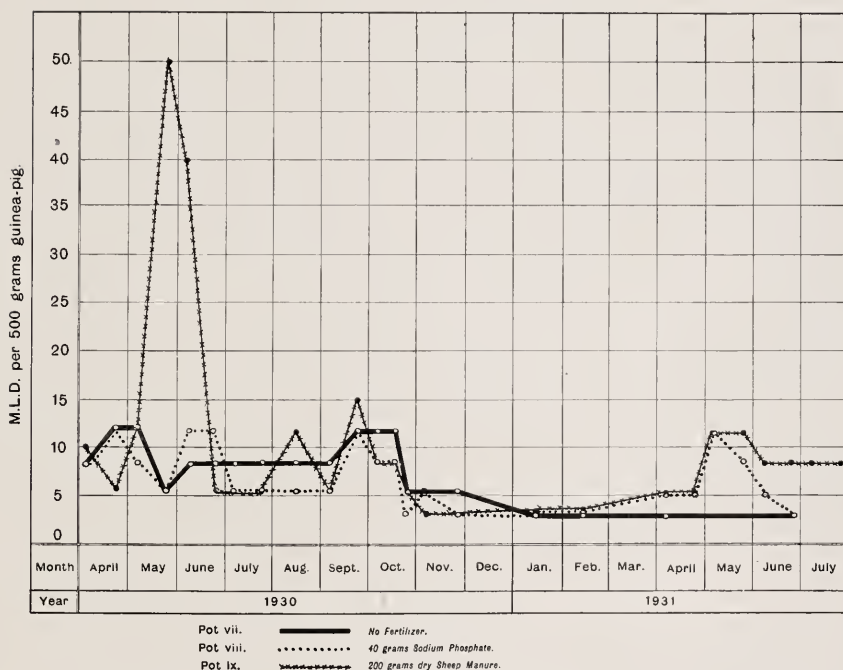
From Graph I it will be seen that within a comparatively short time after transplantation all three specimens showed a decrease in toxicity which was most striking in the case of the sodium phosphate plant, and least pronounced in the case of the sheep manure plant. Whether or not this decrease in toxicity was due to transplantation cannot be definitely stated. It is of interest to note that during the period February to May these three specimens which had in the meantime been increasing in toxicity, again showed a decrease. All three specimens showed the highest degree of toxicity during the period November to January.

Graph II.—In Diffused Sunlight (Black Clay Soil).



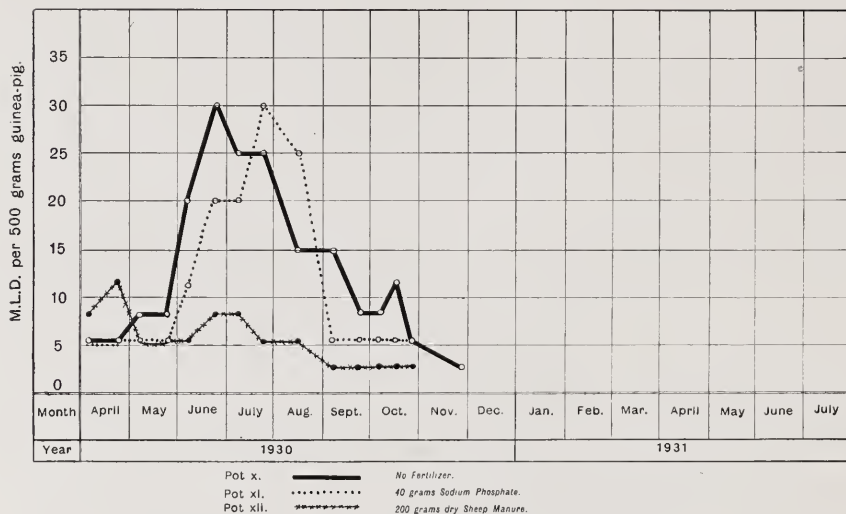
From Graph II it will be noticed that after transplantation there was a decrease in the toxicity of these plants similar to that shown in Graph I, and also that the highest toxicity was reached during November as was the case with the three plants growing in the direct sunlight (Graph I). Unfortunately the tests with the plants growing in diffused sunlight could not be continued as these plants died.

Graph III.—In Direct Sunlight (Sandy Soil).



Graph III shows marked differences in the course of the toxicity of the plants as compared with the two previous graphs. The unfertilized plant showed a slight preliminary decrease in toxicity followed by an increase, which, excepting another slight decrease during September and October, persisted throughout the experiment. The course of the toxicity of the sodium phosphate plant was very similar to that of the unfertilized specimen with the exception of the latter part of the course. The sheep manure plant showed a preliminary increase followed by a pronounced decrease in toxicity, which in the course of the following month again increased considerably.

Graph IV.--In Diffused Light (Sandy Soil).

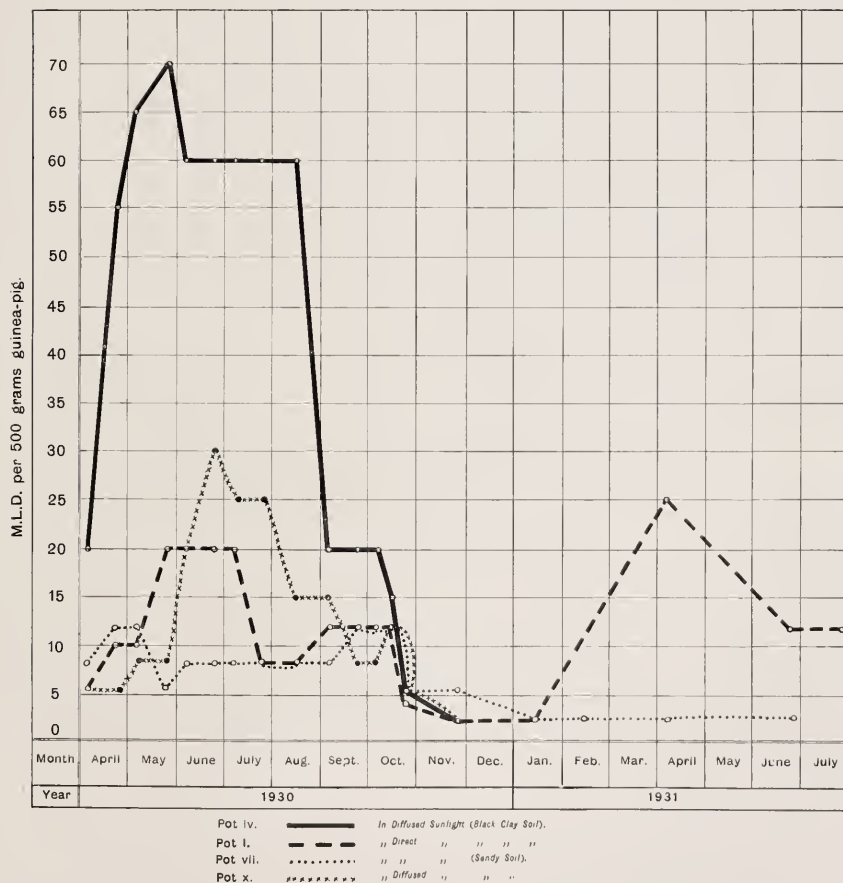


Graph IV shows a striking resemblance between the courses of toxicity of the unfertilized and the sodium phosphate plant, both showing a marked decrease after transplantation whereas the sheep manure plant showed a slight preliminary decrease in toxicity, which was followed by a fairly striking increase. These plants also showed the highest degree in toxicity during October and November. Unfortunately the death of the plants prevented further tests.

It was thought advisable also to prepare three graphs comparing the courses of toxicity of the black clay soil and sandy soil plants without fertilizers, with sodium phosphate and with sheep manure respectively.

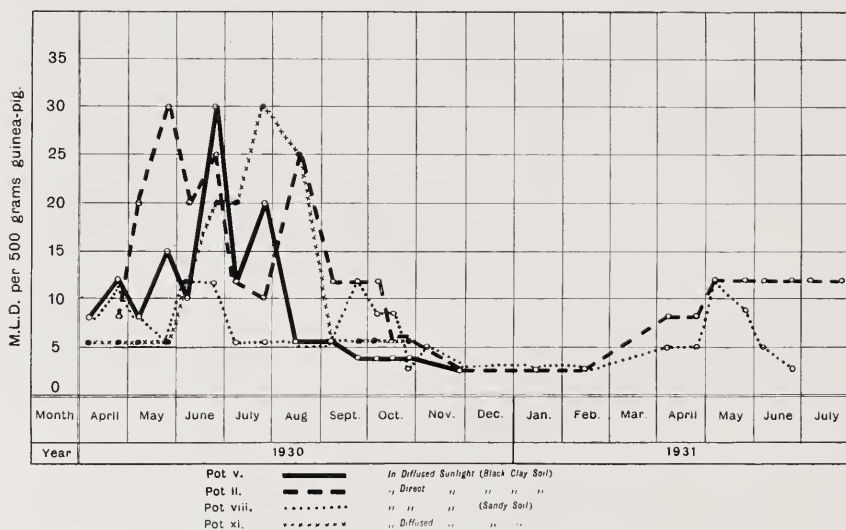
The only noteworthy point in Graph V is that the unfertilized plant growing in black clay soil and in direct sunlight showed a decrease in toxicity during the latter part of the experiment while the specimen in the unfertilized sandy soil kept in direct sunlight showed a marked increase in toxicity.

Graph V.—No Fertilizer.



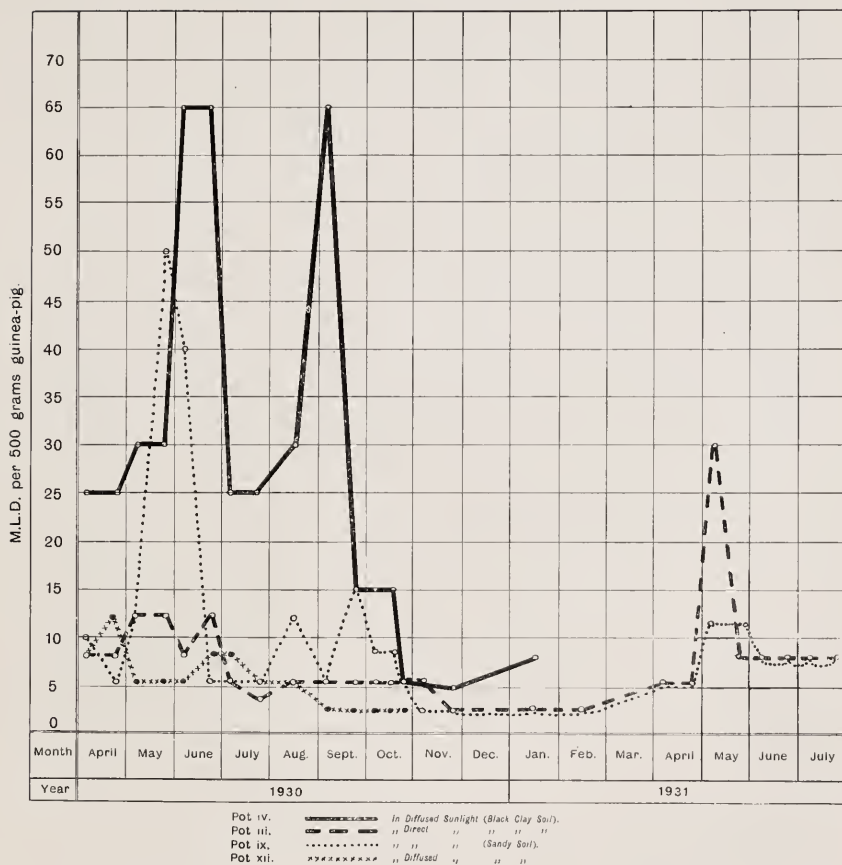
Unfortunately the toxicity of the black clay soil and sandy soil plants growing in diffused sunlight could not be traced to the end of the experiment as they died in November.

Graph VI.—40 Grams Sodium Phosphate.



Also from Graph VI it is evident that the black clay soil specimen growing in direct sunlight was less toxic during the latter part of the experiment than it was on the day the experiment was begun, while in the case of the sandy soil specimen the reverse was true.

Graph VII.—200 Grams Dry Sheep Manure.



It will be noticed from Graph VII that the toxicity of the black clay soil and sandy soil specimens growing in the direct sunlight was identical during the last two months of the experiment, and that the black clay soil specimen was just as toxic at the end as at the beginning of the experiment, while the toxicity of the sandy soil specimen showed a slight increase as compared with the degree of toxicity when the experiment was begun.

DISCUSSION.

(A) LITERATURE.

From the literature it appears that nothing definite about the factors influencing the degree of toxicity of poisonous and medicinal plants is known. Burman found the alkaloidal content of medicinal plants growing in the same locality at its lowest in years of low temperature and deficient sunshine. The alkaloidal content of *Atropa belladonna* could not be increased by means of fertilizers, but full exposure to sunlight appeared to increase the active principle content (Hansom and Henderson). Furthermore, inconclusive results as to the effect of farmyard manure, nitrate, calcium cyanamide, basic slag, superphosphate and potash on the alkaloidal content of *Atropa belladonna* were obtained.

According to Marais the fertility of the soil exerts a striking influence on the palatability of vetches. He found that the vetches produced no nodules when grown in poor soil with a low humus content.

Wheat when grown on soil fertilized with cattle manure has a higher vitamin A content, than when grown on soil treated with complete chemical manure. On the other hand the vitamin B content of millet is higher when grown on soil manured with cattle manure than when grown on soil fertilized with complete chemical manure (McCarrison).

Khalil found that drying causes very pronounced changes in the microbiological processes in soils. It therefore appears that climatic conditions play an important part in determining the toxicity of plants, and that soil conditions influence the palatability and vitamin content of certain crops. Practically nothing is known about the climatic and soil conditions influencing the degree of toxicity of plants, but probably the following factors are concerned, to a certain extent at least, in determining the degree of toxicity of plants, composition and character of the soil, bacterial and protozoal content of the soil, moisture content of the soil and air, sunlight and the atmospheric and soil temperature.

The more porous a soil the better the aeration and consequently one would expect more active oxidative and other processes than in less porous soils. In the microbiological processes in dried and moist soil Khalil found very striking differences, for example, organic matter in dried soil was more rapidly ammonified and nitrified than that in moist soil.

(B) ONDERSTEEPOORT EXPERIMENTS.

It was found (a) that the more finely minced the *Cotyledon* leaves the higher the extractability of the poison, and that the degree of agitation of the *Cotyledon*—96 per cent. alcoholic suspension affected the extractability of the toxin; (b) that immature and mature leaves are equally toxic; (c) that leaves collected from different parts of the same *Cotyledon* plant do not vary in toxicity; and (d) that *Cotyledon orbiculata* specimens growing beside each other vary to a considerable degree in toxicity.

The fact that plants growing beside each other very considerably in toxicity renders it essential that the study of the factors concerned in the determination of the toxicity of plants should be conducted with one and the same plant. If plots of plants be utilized for this purpose and portions of these plots tested at intervals unreliable results will be obtained as the different plants on one and the same plot may vary considerably in toxicity. This method of investigation naturally is possible only with plants of a fair size so as to allow of the picking of test-material from one and the same plant at definite intervals. If the toxicity of a plant of a small size is to be studied it would seem essential that plots of such plants be experimented with and that for each test material be collected from all the individual plants growing on this particular plot in order to determine the "mean toxicity" of the plant on the plot.

(C) EFFECT OF SUNLIGHT.

In regard to the effect of sunlight on the toxicity of *Cotyledon orbiculata* no definite conclusions can be drawn as the specimens growing in the diffused sunlight died after a certain period. Direct sunlight seems to be essential for the normal growth of *Cotyledon orbiculata*, whereas *C. ventricosa* and *C. wallichii* prefer diffused sunlight, as they are found growing under bushes and shrubs only.

Apparently sunlight stimulates the production of atropine in *Atropa belladonna* as according to Burmann the alkaloidal content of this plant is at its lowest in years of low temperature and deficient sunshine, and Ransom and Henderson reported that full exposure to sunlight appears to increase the alkaloidal content of *Atropa belladonna*.

(D) EFFECT OF FERTILIZERS.

The grass collected on the black clay soil utilized in this experiment contained much more P and Ca than the grass collected on the sandy soil. The chemical composition of the soils to be used in future experiments will have to be carefully controlled as it most decidedly will influence the growth of the different plant specimens. Whether minerals play a part in the production of plant-poisons is as yet an open question.

In the experiments at Onderstepoort no definite conclusions could be arrived at in this respect, and it is hoped that future experiments will throw some light on this problem.

All the sodium phosphate and sheep manure specimens showed much better growth than the unfertilized plants (see Figs. V and VI).

(E) PHYSICAL PROPERTIES OF THE LEAVES OF THE DIFFERENT PLANT SPECIMENS.

The leaves of the different specimens and those of the same specimen tested at different times showed marked physical differences when minced and extracted with the alcohol. On mincing the leaves some liberated a large amount of clear fluid, whereas others formed a porridge-like mass with no free fluid or set free a small quantity of a turbid fluid. On mixing the minced leaves with 96 per cent.

alcohol the leaves, which liberated the clear fluid, formed a clear transparent supernatant fluid, whereas those leaves which on mincing formed a porridge-like mass with no or very little free turbid fluid, formed an opaque, sometimes flocculent supernatant fluid.

The evaporated filtrates varied in colour from dark yellow-green to dark pink and the consistence ranged from fluid to extremely mucous.

(F) TOXICITY OF THE DIFFERENT SPECIMENS OF *Cotyledon orbiculata*.

From the comparative table it will be noticed that there was a striking difference in the toxicity of the different specimens utilized in this experiment.

All the specimens, except the sheep manure one growing in sandy soil in the direct sunlight (Pot IX), showed a decrease in toxicity within two weeks to two months after transplantation. The specimen in Pot IX showed an increase in toxicity when tested fourteen days after transplantation, and then followed a striking decrease in toxicity which continued for about a month and then increased. Whether this decrease is toxicity, which was very marked in some specimens after transplantation, was due to the effects of transplantation or not, is being investigated at present.

Again all the specimens kept in the direct sunlight showed the highest toxicity during the period November to February. The six specimens, which had been kept in diffused light showed the highest degree of toxicity during the period of dying, but unfortunately owing to the death of these plants the course of their toxicity could not be traced to the end of the experiment.

From the graphs it is evident that the toxicity of the same plant may at different times vary to a considerable extent (Pots Nos. I, II, III, IV, V, VI, IX, X, XI).

The unfertilized and sodium phosphate specimens growing in direct sunlight in black clay soil were less toxic at the conclusion than at the beginning of the experiment, while the reverse is true of the unfertilized and sodium phosphate specimens growing in direct sunlight and in sandy soil.

The sheep manure specimen growing in direct sunlight and on black clay soil showed the same degree of toxicity at the end as at the beginning of the experiment, while the sheep manure specimen growing in direct sunlight and on sandy soil showed an increase in toxicity as compared with its toxicity at the date of transplantation.

From the above it is clear that the three specimens growing in direct sunlight and on sandy soil, whether fertilized or not, showed a higher toxicity at the end than at the beginning of the experiment, whereas the reverse was true of two of the black clay soil specimens, the toxicity of the third specimen having been the same at the beginning and the conclusion of the experiment.

CONCLUSIONS.

1. The extractability of the poison contained in *Cotyledon orbiculata* leaves depends to a considerable extent on the comminution to which the leaves are reduced before extraction.

2. The degree of agitation of the *Cotyledon*—96 per cent. alcoholic suspension effects the extractability of the toxin.
3. Immature and mature leaves and leaves collected from different parts of the same plant proved to be equally toxic.
4. Specimens of *Cotyledon orbiculata* growing beside each other varied to a considerable extent in toxicity.
5. Sunlight appears to be essential for the growth of *Cotyledon orbiculata*, while in some *Cotyledon* spp. direct sunlight is avoided.
6. Sodium phosphate and sheep manure seemed to stimulate the growth of *Cotyledon orbiculata*.
7. There was a great variation in the physical properties of the leaves of the different specimens and also in the leaves picked from the same plant at different times.
8. The most toxic period of the specimens utilized in this experiment was from November to February.
9. The sandy soil specimens growing in the direct sunlight were more toxic at the conclusion than at the beginning of the experiment, while the black clay soil specimens (except one, which was equally toxic at the beginning and the conclusion of the experiment) growing in the direct sunlight were less toxic at the conclusion than at the commencement of the experiment.

BIBLIOGRAPHY.

- BURMANN, J. (1911). Annual Variation in Potency of Medicinal Plants. *Schweiz. Woch. Chem. Pharm.* Vol. 49, pp. 6-9.
- FRAPS, G. S. (1931). Variations in Vitamin A and in Chemical Composition of Corn. *Texas Agric. Expt. Stn. Bull. No. 422*, March, 1931.
- GHOSH, T. P., and KRISHNA, S. (1930). Jahreszeitliche Veränderungen des Alkaloidsgehaltes der indischen Ephedra-Arten. *Arch. der Pharm. u. Berichte de Deuts. Pharm. Gesellschaft.* Heft. 9, S. 636.
- KAMERMAN, P. (1926). The Toxicity of the Genus *Cotyledon*, the Cause of the Disease Krimpsiekte. *S. Afr. Jnl. of Sc.*, Vol. 23, pp. 185-187.
- KHALIL, F. (1929). The Effect of Drying on the Microbiological Processes in Soils. *Zbl. Bant.* Part 2, Vol. 79, pp. 93-107.
- LONG, H. A. (1917). Plants Poisonous to Live Stock.
- MARAIS, A. (1930). Monthly Report, Division of Extension, Maclear, Cape Province, Union of South Africa. Oct., 1930.
- McCARRISON, R. (1924). The Relation of Manure to the Nutritive and Vitamin Value of certain Grain. *British Med. Jnl.*, Vol. 1, p. 567.
- McCARRISON, R. (1926). The Effect of Manurial Conditions on the Nutritive and Vitamin Values of Millet and Wheat. *Indian Jnl. of Medical Research*, Vol. 14, No. 2, p. 351.
- RANSOM and HENDERSON (1912). "The Effects of Cultivation and Fertilization on the Growth of *Atropa belladonna* and the Alkaloidal Content of the Leaves". *8th Intern. Cong. Appl. Chem.* 1912, Sect. VIII B.
- STEYN, D. G. (1929). Recent Investigations into the Toxicity of Known and Unknown Poisonous Plants in the Union of South Africa. *15th Rept. Dir. Vety. Serv.*, Union of South Africa, pp. 777-804.

APPENDIX.

POT NO. I.—(TABLE 1.)

IN DIRECT SUNLIGHT.

Containing black clay soil as found in the old poisonous plant garden at Onderstepoort.

Date of Watering.	Stage of Growth at Date of Test.	Water Content of Leaves.	M.L.D. per 500 gm. Guinea-pig before Trans-plantation.	Date of Trans-plantation.	M.L.D. per 500 gm. Guinea-pig after Trans-plantation.	Date of Test.	Remarks.
		%	gm.		gm.		
14.4.30	Post seeding stage.....	94	—	—	—	14.4.30	
30.4.30	Small buds just appearing	94.7	—	—	10	30.4.30	
30.5.30	Flower in stalk = 4.5 cm. long	94.2	—	—	10	14.5.30	
25.6.30	Flower and stalk = 8.5 cm. long	94.4	—	—	20	28.5.30	
6.7.30	Flower and stalk = 13.5 cm. long	94	—	—	20	11.6.30	
4.8.30	Flower and stalk = 20 cm. long	94	—	—	20	25.6.30	
13.9.30	Flower and stalk = 25 cm. long	94	—	—	20	9.7.30	
12.10.30	Flower and stalk = 30 cm. long	94	—	—	8	23.7.30	
20.10.30	—	—	—	14.4.30	Not tested	6.8.30	
28.10.30	Shedding ripe seeds	94	—	—	8	20.8.30	
14.11.30	All ripe seeds shedded ; flowerstalk dying	94	—	—	12	3.9.30	
30.11.30	Flowerstalk completely dead. New leaves appearing	94	6	—	12	17.9.30	
15.12.30	More new leaves appearing	94	—	—	12	1.10.30	
30.12.30	„ „	94.8	—	—	12	15.10.30	
20.1.31	„ „	94.6	—	—	4	29.10.30	
15.2.31	—	—	—	—	Not tested	12.11.30	Insufficient leaves.
15.3.31	More new leaves appearing	94.5	—	—	3	26.11.30	
20.4.31	„ „	94.5	—	—	3	14.1.31	
4.5.31	—	—	—	—	Not tested	18.2.31	Insufficient leaves.
18.5.31	More new leaves appearing	94.5	—	—	25	15.4.31	
2.6.31	—	—	—	—	Not tested	29.4.31	Insufficient leaves.
23.6.31	—	—	—	—	„	13.5.31	„
15.7.31	—	—	—	—	„	27.5.31	„
—	More new leaves appearing	94	—	—	„	10.6.31	„
					12 gm.	24.6.31	
					Not tested	8.7.31	Insufficient leaves.
					12 gm.	12.7.31	

POT No. 11.—(TABLE 2.)

IN DIRECT SUNLIGHT.

Containing black clay soil as found in the old poisonous plant garden at Onderstepoort + 40 grams of sodium phosphate.

Date of Watering.	Stage of Growth at Date of Test.	Water Content of Leaves.	M.L.D. per 500 gm. Guinea-pig before Trans-plantation.	Date of Trans-plantation.	M.L.D. per 500 gm. Guinea-pig after Trans-plantation.	Date of Test.	Remarks.
		%	gm.		gm.		
14.4.30	Post seeding stage.....	93·8	—	—	—	14.4.30	
30.4.30	Small buds just appearing	93·6	—	—	8	30.4.30	
30.5.30	Flower and stalk = 8 cm.	93·6	—	—	20	14.5.30	
25.6.30	Flower and stalk = 14 cm.	93	—	—	30	28.5.30	
6.7.30	Flower and stalk = 20 cm.	93	—	—	20	11.6.30	
4.8.30	Flower and stalk = 20 cm.	93	—	—	25	25.6.30	
13.9.30	Flower and stalk = 25 cm.	93	—	—	12	9.7.30	
12.10.30	Flower and stalk = 25 cm.	93	—	—	10	23.7.30	
20.10.30	—	—	—	—	Not tested	6.8.30	
28.10.30	Shedding ripe seeds.....	93	—	—	25	20.8.30	
14.11.30	All ripe seeds shedded	93	—	—	12	3.9.30	
30.11.30	Flowerstalk dying						
30.11.30	Flowerstalk dead. New leaves appearing	92·7	8	14.4.31	12	17.9.30	
15.12.30	More new leaves appearing	93	—	—	12	1.10.30	
30.12.30	“ “	93	—	—	6	15.10.30	
20.1.31	“ “	93	—	—	6	29.10.30	
15.2.31	—	—	—	—	Not tested	12.11.30	Insufficient leaves.
15.3.31	More new leaves appearing	93	—	—	3	26.11.30	
20.4.31	“ “	93	—	—	3	14.1.31	
4.5.31	“ “	92·7	—	—	3	18.2.31	
18.5.31	“ “	93	—	—	8	15.4.31	
2.6.31	“ “	92·8	—	—	8	29.4.31	
23.6.31	“ “	93	—	—	12	13.5.31	
15.7.31	“ “	93	—	—	12	27.5.31	
	“ “	93	—	—	12	10.6.31	
	“ “	93	—	—	12	24.6.31	
	“ “	93	—	—	12	8.7.31	
	“ “	93	—	—	12	22.7.31	

POT No. III.—(TABLE 3.)

IN DIRECT SUNLIGHT.

Containing black clay soil as found in the old poisonous plant garden at Onderstepoort + 200 grams of dry sheep manure.

Date of Watering.	State of Growth at Date of Test.	Water Content of Leaves.	M.L.D. per 500 gm. Guinea-pig before Trans-plantation.	Date of Trans-plantation.	M.L.D. per 500 gm. Guinea-pig after Trans-plantation.	Date of Test.	Remarks.
		%	gm.		gm.		
14.4.30	Post seeding stage.....	93.7	—	—	—	14.4.30	
30.4.30	„ „	92.4	—	—	8	30.4.30	
30.5.30	Post seeding stage (no buds appear)	92.2	—	—	12	14.5.30	
25.6.30	Post seeding stage.....	92.7	—	—	12	28.5.30	
6.7.30	„ „	93.2	—	—	8	11.6.30	
4.8.30	„ „	92.5	—	—	12	25.6.30	
13.9.30	„ „	93	—	—	6	9.7.30	
12.10.30	„ „	93	—	—	4	23.7.30	
20.10.30	—	—	—	—	Not tested	6.8.30	
28.10.30	Post seeding stage.....	92.5	—	—	6	20.8.30	
14.11.30	„ „	94	—	—	6	3.9.30	
30.11.30	New leaves appearing..	93.5	—	—	6	17.9.30	
15.12.30	More new leaves appearing	93.6	8	14.4.30	6	1.10.30	
30.12.30	„ „ ..	94.3	—	—	6	15.10.30	
20.1.31	„ „ ..	94.5	—	—	6	29.10.30	
15.2.31	„ „ ..	94	—	—	6	12.11.30	
15.3.31	„ „ ..	94	—	—	3	26.11.30	
20.4.31	„ „ ..	94	—	—	3	14.1.31	
4.5.31	„ „ ..	93.8	—	—	3	18.2.31	
18.5.31	„ „ ..	94	—	—	6	15.4.31	
2.6.31	„ „ ..	94	—	—	6	29.4.31	
23.6.31	„ „ ..	93.8	—	—	30	13.5.31	
15.7.31	„ „ ..	94	—	—	8	27.5.31	
	„ „ ..	94	—	—	8	10.6.31	
	„ „ ..	94	—	—	8	24.6.31	
	„ „ ..	94	—	—	8	8.7.31	
	„ „ ..	94	—	—	8	22.7.31	

POT NO. IV.—(TABLE 4.)

IN DIFFUSED LIGHT.

Containing black clay soil as found in the old poisonous plant garden at Onderstepoort.

Date of Watering.	Stage of Growth at Date of Test.	Water Content of Leaves.	M.L.D. per 500 gm. Guinea-pig before Transplantation.	Date of Transplantation.	M.L.D. per 500 gm. Guinea-pig after Transplantation.	Date of Test.	Remarks.
		%	gm.		gm.		
14.4.30	Post seeding stage.....	94.8	—	—	—	14.4.30	
30.4.30	Small buds just appearing	94.6	—	—	55	30.4.30	
30.5.30	Flower and stalk = 6 cm.	94.5	—	—	65	14.5.30	
25.6.30	Flower and stalk = 12 cm.	94.1	—	—	70	28.5.30	
6.7.30	Flower and stalk = 17 cm.	94.5	—	—	65	11.6.30	
4.8.30	Flower and stalk = 24 cm.	95	—	—	65	25.6.30	
13.9.30	Flower and stalk = 32 cm.	94.5	—	—	65	9.7.30	Flowerheads abnormally small and dropped off before maturing.
12.10.30	All flowerheads dropped off. Stalk dying	95	—	—	65	23.7.30	
20.10.30	—	—	—	—	Not tested	6.8.30	
28.10.30	Flowerstalk dead.....	96.4	—	—	65	20.8.30	
14.11.30	New leaves appearing..	97.4	—	—	20	3.9.30	
30.11.30	More new leaves appearing	95.1	—	—	20	17.9.30	
15.12.30	” ”	95.1	—	—	20	1.10.30	
30.12.30	” ”	96.4	20	14.4.30	15	15.10.30	
20.1.30	” ”	96.4	—	—	6	29.10.30	
		96	—	—	Not tested	12.11.30	Insufficient leaves.
					3	26.11.30	
					Not tested	14.1.31	Plant dying — no leaves left.
						18.2.31	Completely dead.

POT No. V.—(TABLE 5.)

IN DIFFUSED LIGHT.

Containing normal black clay soil as found in the old poisonous plant garden at Onderstepoort + 40 grams of sodium Phosphate.

Date of Watering.	Stage of Growth at Date of Test.	Water Content of Leaves.	M.L.D. per 500 gm. Guinea-pig before Transplantation.	Date of Transplantation.	M.L.D. per 500 gm. Guinea-pig after Transplantation.	Date of Test.	Remarks.
		%	gm.		gm.		
14.4.30	Post seeding stage.....	94.4	—	—	—	14.4.30	
30.4.30	Small buds appearing...	95.2	—	—	12	30.4.30	
30.5.30	Flower and stalk = 11 cm.	94.0	—	—	8	14.5.30	
25.6.30	Flower and stalk = 30 cm.	95.4	—	—	15	28.5.30	
6.7.30	Flower and stalk = 32 cm.	96.1	—	—	10	11.6.30	
4.8.30	Flower and stalk = 41 cm.	97	—	—	30	25.6.30	
13.9.30	Flower and stalk = 52 cm.	97	—	—	12	9.7.30	Flowerheads abnormally small and dropped off before maturity.
12.10.30	Flowerstalk dying.....	96.9	—	—	20	23.7.30	
20.10.30	—	—	—	—	Not tested	6.8.30	
28.10.30	Flowerstalk dead.....	97.2	—	—	6	20.8.30	
14.11.30	New buds and new leaves appearing	97	—	—	6	3.9.30	
30.11.30	Flower and flowerstalk = 10 cm. New leaves appearing	—	—	—	4	17.9.30	Flowerheads abnormally small and dropped off before maturity.
15.12.30	Flowerheads all dropped off. New leaves appearing	95	—	—	4	1.10.30	
30.12.30	More new leaves appearing	97.5	—	—	4	15.10.30	
20.1.31	“ “	97	8	14.4.30	4	29.10.30	
15.2.31	—	—	—	—	Not tested	12.11.30	Insufficient material.
		97	—	—	3	26.11.31	Plant dying.
						14.1.31	Completely dead.

POT No. VI.—(TABLE 6.)

IN DIFFUSED LIGHT.

Containing black clay soil as found in the old poisonous plant garden at Onderstepoort + 200 grams of dry sheep manure.

Date of Watering.	State of Growth at Date of Test.	Water Content of Leaves.	M.L.D. per 500 gm. Guinea-pig before Trans-plantation.	Date of Trans-plantation.	M.L.D. per 500 gm. Guinea-pig after Trans-plantation.	Date of Test.	Remarks.
		%	gm.		gm.		
14.4.30	Post seeding stage.....	93.7	—	—	—	14.4.30	
30.4.30	Buds just appearing....	94.9	—	—	25	30.4.30	
30.5.30	Flower and stalk = 7.5 cm.	94.6	—	—	30	14.5.30	
25.6.30	Flower and stalk = 16 cm.	94.6	—	—	30	28.5.30	
6.7.30	Flower and stalk = 24 cm.	95.5	—	—	56	11.6.30	
4.8.30	Flowerstalk = 38 cm...	96	—	—	65	25.6.30	
13.9.30	Flower and stalk = 31 cm.	96	—	—	25	9.7.30	Flowerheads abnormally small and dropped off before maturity.
12.10.30	Flowerstalk dying.....	96	—	—	25	23.7.30	
20.10.30	—	—	—	—	Not tested	6.8.30	
28.10.30	New leaves appearing..	96	—	—	30	20.8.30	
14.11.30	More new leaves appearing	96	—	—	65	3.9.30	
30.11.30	" "	96	—	14.4.30	15	17.9.30	
15.12.30	" "	95.5	—	—	15	1.10.30	
30.12.30	" "	95	25	—	15	15.10.30	
20.1.31	" "	95	—	—	6	29.10.30	
15.2.31	—	—	—	—	Not tested	12.11.30	Insufficient material.
	More new leaves appearing	94.7	—	—	5	26.11.30	
	Plant dying.....	95	—	—	8	14.1.31	Plant dying.
						18.2.31	Plant almost dead no leaves left, moved in direct sunlight. Within a week a number of normal leaves appeared, but died off after two months.

POT No. VII.—(TABLE 7.)

IN DIRECT SUNLIGHT.

Containing sandy soil as found in the new poisonous plant garden at Onderstepoort.

Date of Watering.	Stage of Growth at Date of Test.	Water Content of Leaves.	M.L.D. per 500 gm. Guinea-pig before Trans-plantation.	Date of Trans-plantation.	M.L.D. per 500 gm. Guinea-pig after Trans-plantation.	Date of Test.	Remarks.
		%	gm.		gm.		
4.4.30	Post seeding stage.....	93.6	—	—	—	14.4.30	
30.4.30	Post seeding stage.....	93.5	—	—	12	30.4.30	
30.5.30	Flowers and stalk = 3.5 cm.	94	—	—	12	14.5.30	
25.6.30	Flowers and stalk = 4.5 cm.	93.2	—	—	6	28.5.30	
6.7.30	Flowers and stalk = 14 cm.	92	—	—	8	11.6.30	
4.8.30	Flowers and stalk = 20 cm.	92.4	—	—	8	25.6.30	
13.9.30	Flowers and stalk = 27 cm.	92	—	—	8	9.7.30	
12.10.30	Flowers and stalk = 37 cm.	92	—	—	8	23.7.30	
20.10.30	—	—	—	—	Not tested	6.8.30	
28.10.30	Seeds maturing, new appearing	92.8	—	—	8	20.8.30	
14.11.30	Seeds being shed, etc...	93.3	—	—	8	3.9.30	
30.11.30	All seeds shed. New leaves, etc.	93.6	8	14.4.30	12	17.9.30	
15.12.30	New leaves appearing...	93.8	—	—	12	1.10.30	
30.12.30	" " ...	92.9	—	—	12	15.10.30	
20.1.31	" " ...	93	—	—	6	29.10.30	
15.2.31	—	—	—	—	Not tested	12.11.30	Insufficient material.
15.3.31	New leaves appearing...	93	—	—	6	26.11.30	
20.4.31	" " ...	93	—	—	3	14.1.31	
4.5.31	" " ...	92.8	—	—	3	18.2.31	
18.5.31	" " ...	92.6	—	—	3	15.4.31	
2.6.31	—	—	—	—	Not tested	29.4.31	Insufficient material.
23.6.31	—	—	—	—	"	13.5.31	" "
15.7.31	—	—	—	—	"	27.5.31	" "
					"	10.6.31	" "
	New leaves appearing...	93	—	—	3	24.6.31	
					Not tested	8.7.31	Insufficient material.
					"	22.7.31	" "

POT No. VIII.—(TABLE 8.)

IN DIRECT SUNLIGHT.

Containing sandy soil as found in the new poisonous plant garden at Onderstepoort + 40 grams of sodium phosphate.

Date of Watering.	Stage of Growth at Date of Test.	Water Content of Leaves.	M.L.D. per 500 gm. Guinea-pig before Trans-plantation.	Date of Trans-plantation.	M.L.D. per 500 gm. Guinea-pig after Trans-plantation.	Date of Test.	Remarks.
		%	gm.		gm.		
4.4.30	Post seeding stage.....	93	—	—	—	14.4.30	
30.4.30	" "	92	—	—	12	30.4.30	
30.5.30	" "	94	—	—	8	14.5.30	
25.6.30	" "	93.6	—	—	6	28.5.30	
6.7.30	Flowers and stalk = 4.5 cm.	92.3	—	—	12	11.6.30	
4.8.30	Flowers and stalk = 10 cm.	92.8	—	—	12	25.6.30	
13.9.30	Flowers and stalk = 13 cm.	93	—	—	6	9.7.30	
12.10.30	Flowers and stalk = 15 cm.	93.2	—	—	6	23.7.30	
20.10.30	—	—	—	—	Not tested	6.8.30	
28.10.30	Seeds ripening and being shed	93.4	8	—	6	20.8.30	
14.11.30	Seeds shed. New leaves appearing	93.7	—	—	6	3.9.30	
30.11.30	New leaves appearing...	93.5	—	—	12	17.9.30	
15.12.30	" " ..	93.5	—	14.4.30	8	1.10.30	
30.12.30	" " ..	93.6	—	—	8	15.10.30	
20.1.31	" " ..	94	—	—	3	29.10.30	
15.2.31	" " ..	94	—	—	6	12.11.30	
15.3.31	" " ..	94	—	—	3	26.11.30	
20.4.31	" " ..	94.5	—	—	3	14.1.31	
4.5.31	" " ..	94	—	—	3	18.2.31	
18.5.31	" " ..	94	—	—	5	15.4.31	
2.6.31	" " ..	94	—	—	5	29.4.31	
23.6.31	" " ..	94	—	—	12	15.5.31	
15.7.31	" " ..	94	—	—	8	27.5.31	
	" " ..	94	—	—	5	10.6.31	
	" " ..	93.8	—	—	3	24.6.31	
					Not tested	8.7.31	Insufficient material.
					Not tested	22.7.31	"

POT No. IX.—(TABLE 9.)

IN DIRECT SUNLIGHT.

Containing sandy soil as found in the new poisonous plant garden at Onderstepoort + 200 grams of dry manure.

Date of Watering.	Stage of Growth at Date of Test.	Water Content of Leaves.	M.L.D. per 500 gm. Guinea-pig before Trans-plantation.	Date of Trans-plantation.	M.L.D. per 500 gm. Guinea-pig after Trans-plantation.	Date of Test.	Remarks.
		%	gm.		gm.		
14.4.30	Post seeding stage.....	91.7	—	—	—	14.4.30	
30.4.30	Buds just appearing.....	93.8	—	—	6	30.4.30	
30.5.30	Flowers and stalk = 4 cm.	90.8	—	—	12	14.5.30	
25.6.30	Flowers and stalk = 9 cm.	91.5	—	—	50	28.5.30	
6.7.30	Flowers and stalk = 18 cm.	93.8	—	—	40	11.6.30	
4.8.30	Flowers and stalk = 25 cm.	93.8	—	—	6	25.6.30	
13.9.30	Flowers and stalk = 27 cm.	94	—	—	6	9.7.30	
12.10.30	Flowers and stalk = 30 cm.	94.3	—	—	6	23.7.30	
20.10.30	—	—	—	—	Not tested	6.8.30	
28.10.30	Ripe seeds being shed	94.1	10	—	12	20.8.30	
14.11.30	94.5	—	—	6	3.9.30	
30.11.30	New leaves appearing..	93	—	—	15	17.9.30	
15.12.30	93	—	—	8	1.10.30	
30.12.30	93.6	—	14.4.30	8	15.10.30	
20.1.31	93.5	—	—	6	29.10.30	
15.2.31	93.5	—	—	3	12.11.30	
15.3.31	93.5	—	—	3	26.11.30	
20.4.31	93	—	—	3	14.1.31	
4.5.31	93.5	—	—	3	18.2.31	
18.5.31	94*	—	—	5	15.4.31	
2.6.31	94	—	—	5	29.4.31	
23.6.31	94	—	—	12	15.5.31	
15.7.31	93.8	—	—	12	27.5.31	
	94	—	—	8	10.6.31	
	94	—	—	8	24.6.31	
	94	—	—	8	8.7.31	
	94	—	—	8	22.7.31	

POT No. X.—(TABLE 10.)

IN DIFFUSED LIGHT.

Containing sandy soil as found in the new poisonous plant garden at Onderstepoort.

Date of Watering.	Stage of Growth at Date of Test.	Water Content of Leaves.	M.L.D. per 500 gm. Guinea-pig before Transplantation.	Date of Transplantation.	M.L.D. per 500 gm. Guinea-pig after Transplantation.	Date of Test	Remarks.
		%	gm.		gm.		
14.4.30	Post seeding stage.....	94.6	—	—	—	14.4.30	
30.4.30	Buds just appearing...	94.6	—	—	6	30.4.30	
30.5.30	Flowers and stalk = 5 cm.	94.5	—	—	8	14.5.30	
25.6.30	Flower and stalk = 13 cm.	94.4	—	—	8	28.5.30	
6.7.30	Flower and stalk = 16 cm.	94.4	—	—	20	11.6.30	
4.8.30	Flower and stalk = 26 cm.	95	—	—	30	25.6.30	
13.9.30	Flower and stalk = 49 cm.	95	—	—	25	9.7.30	
12.10.30	All flowerheads dropped off in an immature state	95.5	6	14.4.30	25	23.7.30	
20.10.30	—	—	—	—	Not tested	6.8.30	
28.10.30	Flower stalk dead.....	95.7	—	—	15	20.8.30	
14.11.30	New leaves appearing..	96	—	—	15	3.9.30	
30.11.30	" " ..	95	—	—	8	17.9.30	
15.12.30	" " ..	95	—	—	8	1.10.30	
30.12.30	" " ..	96	—	—	12	15.10.30	
30.12.30	" " ..	96	—	—	6	29.10.30	
30.12.30	" " ..	—	—	—	Not tested	12.11.30	Insufficient material, plant dying off.
		96	—	—	3	26.11.30	Plant dying off.
						14.1.31	Plant completely dead.

POT No. XI.—(TABLE II.)

IN DIFFUSED LIGHT.

Containing ordinary sandy soil as found in the new poisonous plant garden at Onderstepoort + 40 grams sodium phosphate.

Date of Watering.	State of Growth at Date of Test.	Water Content of Leaves.	M.L.D. per 500 gm. Guinea-pig before Trans-plantation.	Date of Trans-plantation.	M.L.D. per 500 gm. Guinea-pig after Trans-plantation.	Date of Test.	Remarks.
		%	gm.		gm.		
14.4.30	Post seeding stage.....	94.2	—	—	—	14.4.30	
30.4.30	Buds just appearing....	95.3	—	—	6	30.4.30	
30.5.30	Flowers and stalk = 3.5 cm.	94.9	—	—	6	14.5.30	
25.6.30	Flowers and stalk = 11 cm.	94.9	—	—	6	28.5.30	
6.7.30	Flowers and stalk = 11 cm.	94.7	—	—	12	11.6.30	
4.8.30	Flowers and stalk = 16 cm.	95	—	—	20	25.6.30	
13.9.30	Flowers and stalk = 27 cm.	96	—	—	20	9.7.30	
12.10.30	All flowerheads dropped off in an immature state	96	—	—	30	23.7.30	
20.10.30	—	—	6	14.4.30	Not tested	6.8.30	
28.10.30	Flowerstalk dead. New leaves appearing	96.2	—	—	25	20.8.30	
14.11.30	New leaves appearing..	96.3	—	—	6	3.9.30	
30.11.30	" " "	96.3	—	—	6	17.9.30	
15.12.30	" " "	95.7	—	—	6	1.10.30	
30.12.30	" " "	95.4	—	—	6	15.10.30	
		95.6	—	—	6	29.10.30	
					Not tested	12.11.30	Plant dying off.
						26.11.30	Plant dying off, completely dead.

POT NO. XII.—(TABLE 12.)

IN DIFFUSED LIGHT.

Containing ordinary sandy soil as found in the new poisonous plant garden at Onderstepoort + 200 grams dry sheep manure.

Date of Watering.	Stage of Growth at Date of Test.	Water Content of Leaves.	M.L.D. per 500 gm. Guinea-pig before Trans-plantation.	Date of Trans-plantation.	M.L.D. per 500 gm. Guinea-pig after Trans-plantation.	Date of Test.	Remarks.
		%	gm.		gm.		
14.4.30	Post seeding stage.....	92.9	—	—	—	14.4.30	
30.4.30	Post seeding stage.....	93.4	—	—	12	30.4.30	
30.4.30	Buds just appearing....	95.2	—	—	6	14.5.30	
25.6.30	Flowers and stalk = 2 cm.	94.8	—	—	6	28.5.30	
6.7.30	Flowers and stalk = 4 cm.	95	—	—	6	11.6.30	
4.8.30	Flowers and stalk = 5 cm.	95	—	14.4.30	8	25.6.30	
13.9.30	Flowers and stalk = 10 cm.	95	—	—	8	9.7.30	
12.10.30	Flowerheads dying off, in an immature stage	95	—	—	6	23.7.30	
20.10.30	—	—	—	—	Not tested	6.8.30	
28.10.30	Flowerstalk dead. New leaves appearing	95.2	8	—	6	20.8.30	
14.11.30	New leaves appearing..	95	—	—	3	3.9.30	
30.11.30	" " ..	94.8	—	—	3	17.9.30	
15.12.30	" " ..	95.1	—	—	3	1.10.30	
30.12.30	" " ..	95	—	—	3	15.10.30	
	" " ..	95	—	—	3	29.10.30	
					Not tested	12.11.30	Insufficient leaves.
						26.11.30	Plant dying.
						14.1.31	Plant completely dead.

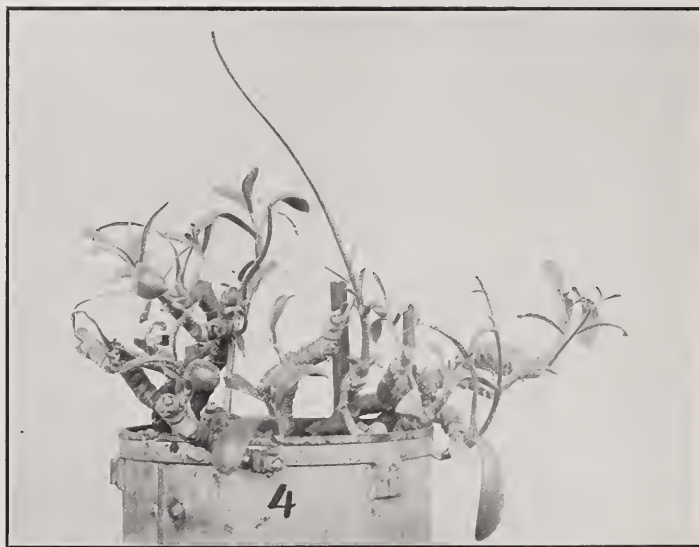


Fig. 1.—In diffused sunlight (24.10.30).
4.—Black clay soil.

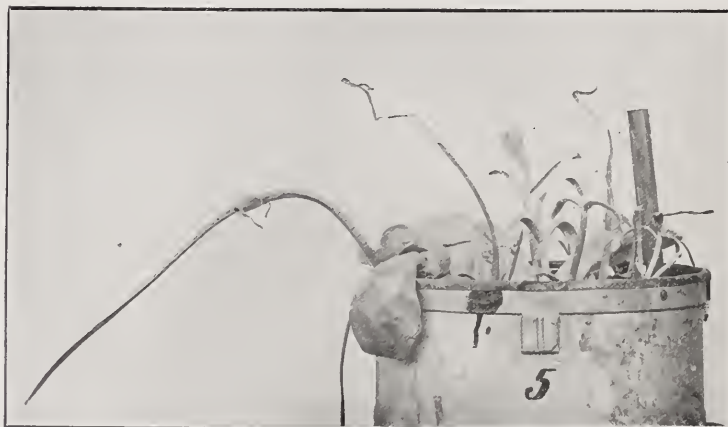


Fig. II.—In diffused sunlight (24.10.30).
5.—Black clay soil + Sodium phosphate.



Fig. III.—In diffused sunlight (24.10.30).
6.—Black clay soil + Sheep manure.
10.—Sandy soil.



Fig. IV.—In diffused sunlight (24.10.30).
11.—Sandy soil + Sodium phosphate.
12.—Sandy soil + Sheep manure.



Fig. V.—In direct sunlight (24.10.30).

- 1.—Black clay soil.
- 2.—Black clay soil + Sodium phosphate.
- 3.—Black clay soil + Sheep manure.



Fig. VI.—In direct sunlight (24.10.30).

- 7.—Sandy soil.
- 8.—Sandy soil + Sodium phosphate.
- 9.—Sandy soil + Sheep manure.

Experiments with Potassium Cyanide on Rabbits.

By D. G. STEYN, B.Sc., Dr.Med.Vet., Veterinary Research
Officer, Onderstepoort.

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EXPERIMENT I.

TO DETERMINE WHETHER THERE IS A DIFFERENCE IN THE SUSCEPTIBILITY OF WHITE ANGORA AND COLOURED RABBITS TO POTASSIUM CYANIDE.

IN the course of experiments with various poisonous substances, it appeared that Angora rabbits showed a greater susceptibility than coloured rabbits to these substances. The following instance might serve as an example. In an experiment to determine the M.L.D. of KCN per 2 Kg. rabbit, it was noticed that a dark-grey rabbit exhibited very slight symptoms of poisoning, while an Angora rabbit, which had received the same amount of KCN per 2 Kg. body weight, died within one hour after dosage. As tolerance and idiosyncrasy might have played a part in the cases that came to my notice, it was thought advisable to investigate this matter.

For this purpose full-grown white Angora and dark-grey rabbits were chosen and the effects of KCN tested on them. 0.2 gm. KCN was dissolved in 400 c.c. distilled water and the animals drenched by means of a stomach tube with the requisite amounts of this solution. Each animal received 5 c.c. of tap-water immediately after it had been drenched with the KCN solution in order to prevent any of the solution remaining in the stomach tube. This method of dosing was adopted in all the experiments. Details of the experiment are given below:

The susceptibility of eight white Angora rabbits was compared with that of eight dark-grey rabbits. Five of the eight Angora rabbits died while only two of the dark-grey rabbits succumbed to KCN poisoning. In addition, it will be noticed that the Angora rabbits which recovered had exhibited much more pronounced symptoms than the dark-grey rabbits.

Subsequently the above experiment was repeated with nine dark-grey and nine white Angora rabbits, with the result that two of the former and seven of the latter died. The symptoms exhibited by those dark-grey rabbits, which recovered, were noticeably less severe than those exhibited by the white Angora rabbits, which did not succumb to KCN poisoning.

After it had been established that Angora rabbits possess a higher degree of susceptibility than dark-grey rabbits to KCN, ordinary short-haired white rabbits were tested.

Two groups (A and B) of rabbits, each comprising six short-haired white and six dark-grey rabbits, were engaged in this experiment. In group A one white and one grey rabbit died while in group B a white and two grey rabbits died.

From these experiments there appears to be no difference in the susceptibility of ordinary white and dark-grey rabbits.

The Susceptibility of White Angora and Coloured Rabbits to KCN.

Rabbit No.	Colour.	Weight.	Quantity of KCN given (in gm.).	Quantity of KCN per 2 Kg. Rabbit.	Result.
1	White Angora	1.8 Kg.	0.0135	0.015 gm.	Very pronounced symptoms within 7 minutes. Died within 50 minutes.
2	"	1.8 "	0.0135		Very pronounced symptoms within 10 minutes. Died within 4 hours.
3	"	2.1 "	0.0157		Very pronounced symptoms within 7 minutes. Recovered within 5 hours.
4	"	2.5 "	0.0188		Very pronounced symptoms within 8 minutes. Died within 40 minutes.
5	"	2.15 "	0.0161		Very pronounced symptoms within 15 minutes. Died within 4 hours.
6	"	2.1 "	0.0157		Very pronounced symptoms within 12 minutes. Recovered within 2½ hours.
7	"	2.4 "	0.018		Very pronounced symptoms within 8 minutes. Died within 3 hours.
8	"	1.77 "	0.0133		Very pronounced symptoms within 7 minutes. Recovered within 5 hours.
9	Dark grey	1.6 "	0.012		Very slight symptoms within 10 minutes. Recovered within 30 minutes.
10	"	2.37 "	0.0177		Very pronounced symptoms within 10 minutes. Died within 2 hours.
11	"	2.28 "	0.0171		Very pronounced symptoms within 8 minutes. Died within 1½ hours.
12	"	1.7 "	0.0128		Fairly pronounced symptoms within 15 minutes. Recovered within 1½ hours.
13	"	2.0 "	0.015		Very pronounced symptoms within 10 minutes. Recovered within 5 hours.
14	"	1.82 "	0.0136		Fairly pronounced symptoms within 12 minutes. Recovered within 1¼ hours.
15	"	1.6 "	0.012		Slight symptoms within 10 minutes. Recovered within 30 minutes.
16	"	2.0 "	0.015		Slight symptoms within 10 minutes. Recovered within 2 hours.

DISCUSSION.

It has been definitely proved that the white Angora rabbits utilized in the above experiments were more susceptible than dark-grey rabbits to KCN. Ordinary white short-haired rabbits did not show this increased susceptibility when compared with dark-grey rabbits.

This increased susceptibility of the white Angora rabbits as compared with that of ordinary white and dark-grey rabbits, is most probably due to the fact that the former are of a weaker constitution, which has been brought about by specialised breeding.

This point is of the utmost importance in the determination of the degree of toxicity of plants and other suspected materials. The unreliability of the results is obvious when coloured and ordinary

white rabbits and white Angora rabbits are used in one and the same experiment and in tests to compare the toxicity of different plants.

EXPERIMENT II.

TO DETERMINE WHETHER RABBITS DEVELOP A TOLERANCE TO POTASSIUM CYANIDE.

It must be stated here that striking variations in the M.L.D. of KCN per 2 Kg. rabbit were met with in the course of the experiments with KCN on rabbits. On one occasion it was found that six out of eight rabbits died when dosed with 0.012 gm. KCN per 2 Kg. body weight of rabbit, while at other times 0.014 gm. KCN per Kilogram body weight killed none of the experimental rabbits. Fullgrown dark-grey rabbits, which appeared to be perfectly healthy, were employed in all experiments and post-mortems were conducted on all the animals that died.

Each of eight dark-grey rabbits were dosed as follows:

Date.	Dose of KCN per Kg. Body Weight.	Results.
26.5.31	0.01 gm.	} All rabbits showed slight transient laboured respiration.
27.5.31	"	
28.5.31	"	
29.5.31	"	
30.5.31	"	
2.6.31	0.011 gm.	} " " " "
3.6.31	"	
4.6.31	"	
5.6.31	"	
6.6.31	"	
8.6.31	0.012 gm.	} " " " "
9.6.31	"	
10.6.31	0.013 gm.	} On 10.6.31 only one rabbit showed pronounced laboured respiration, while on 11.6.31 six rabbits exhibited pronounced symptoms of dyspnoea.
11.5.31	"	
12.6.31	0.014 gm.	} On 12.6.31 all rabbits showed pronounced laboured respiration, and very slight dyspnoea on the following day.
13.6.31	"	
15.6.31	0.015 gm.	} On 15.6.31 four rabbits showed pronounced dyspnoea; the following day only one showed laboured respiration, and on 17.6.31 five showed very slight dyspnoea.
16.6.31	"	
17.6.31	"	
18.6.31	0.016 gm.	} On 18.6.31 three rabbits exhibited severe symptoms of dyspnoea; on the following day all rabbits developed very pronounced dyspnoea with paralysis in three cases, and on 20.6.31 the dyspnoea was much less marked, although paralysis occurred in two cases.
19.6.31	"	
20.6.31	"	

CONTROLS.

A. On 12.6.31 seven dark-grey rabbits were dosed with 0.014 gm. KCN per 2 Kg. body weight with the result that five died within $\frac{1}{4}$ hour, $\frac{1}{2}$ hour, $1\frac{1}{4}$ hours, 2 hours and 4 hours respectively.

B. On 20.6.31 six dark-grey rabbits received 0.016 gm. KCN per 2 Kg. body weight. All these rabbits died within 10 minutes, $\frac{1}{4}$ hour, 20 minutes, $\frac{1}{2}$ hour, 40 minutes and 50 minutes respectively.

DISCUSSION.

From the above it will be seen that the experiment was begun by dosing the rabbits with 0.01 gm. KCN per 2 Kg. body weight. This dose caused only very slight transient laboured respiration at varying intervals. The dose of KCN was gradually increased by one-tenth of the original quantity.

The variation in the degree of the symptoms produced in the same animal by the same dose administered on different days was most striking. For example, in quite a number of cases it was found that 0.016 gm. per Kg. body weight caused only slight laboured respiration on the first and third days of dosage, while on the second day it caused most severe symptoms (paralysis).

It was most astonishing how quickly some of the rabbits, that were practically dying, recovered.

On 12.6.31 and 20.6.31 the resistance of the rabbits treated with KCN was compared with that of untreated rabbits. The results appear to indicate that the rabbits, which had been treated with increasing quantities of KCN, had developed a certain degree of tolerance to this poison as compared with the controls.

Hess (1924), who kept pigeons for a period of two months in an atmosphere containing a large amount of hydrocyanic acid, found that these birds had developed no tolerance to this poison.

EXPERIMENT III.

TO DETERMINE THE VALUE OF SULPHUR AS A PREVENTATIVE IN PRUSSIC ACID POISONING IN RABBITS.

The author (1929) has established that sulphur has great value as a preventative in prussic acid poisoning as caused by the ingestion of *Dimorphotheca spectabilis* (bietou). These experiments were conducted with sheep and it was decided to repeat them with rabbits. Potassium cyanide, of which the M.L.D. per 2 Kg. rabbit was found to be approximately 0.015 gm., was utilized as the source of prussic acid. Ten rabbits were employed in these M.L.D. tests.

Each of three groups of two rabbits received 0.5, 1.0 and 3.0 gm. of sulphur respectively on three successive days and their resistance to prussic acid was tested on the third day of the experiment.

It was attempted to dose the animals by placing the sulphur either dry or moistened with water on the back of the tongue, but it was soon realised that the size of the doses could not be accurately controlled, as the animals bluntly refused to swallow the sulphur with the result that wastage occurred. It was then decided to suspend the sulphur in a small quantity of water and to drench the rabbits by means of a stomach tube.

0.2 gram potassium cyanide was dissolved in 400 c.c. of tapwater and the necessary amounts of this solution drenched to the rabbits.

Details of the experiment are given in the following table:

Sulphur as Preventive in KCN Poisoning.

Rabbit No.	Amounts of Sulphur given and Dates of Dosage.	M.L.D. of KCN per 2 Kg. Rabbit.	Amount of KCN given per 2 Kg. Rabbit.	Remarks.
1.	16.4.31, 10 a.m.—0.5 gram S.... 17.4.31, 10 a.m.—0.5 gram S.... 18.4.31, 10 a.m.—0.5 gram S....	{	0.015 gram at 3.10 p.m., 18.4.31	Remained healthy.
2.	16.4.31, 10.10 a.m.—0.5 gram S. 17.4.31, 10.10 a.m.—0.5 gram S. 18.4.31, 10.10 a.m.—0.5 gram S.		0.03 gram at 3.15 p.m., 18.4.31.	Symptoms of prussic acid poisoning appeared within 5 minutes and death within 1 hour after dosage.
3.	16.4.31, 10.20 a.m.—1.0 gram S. 17.4.31, 10.20 a.m.—1.0 gram S. 18.4.31, 10.20 a.m.—1.0 gram S.		0.015 gram at 3.25 p.m., 18.4.31	Remained healthy.
4.	16.4.31, 10.25 a.m.—1.0 gram S. 17.4.31, 10.25 a.m.—1.0 gram S. 18.4.31, 10.25 a.m.—1.0 gram S.		0.03 gram at 3.30 p.m., 18.4.31.	Symptoms of prussic acid poisoning appeared within 5 minutes and death within 1½ hours after dosage.
5.	16.4.31, 10.30 a.m.—3.0 gram S. 17.4.31, 10.30 a.m.—3.0 gram S. 18.4.31, 10.30 a.m.—3.0 gram S.		0.015 gram at 3.35 p.m., 18.4.31	Remained healthy.
6.	16.4.31, 10.35 a.m.—3.0 gram S. 17.4.31, 10.35 a.m.—3.0 gram S. 18.4.31, 10.35 a.m.—3.0 gram S.		0.03 gram at 3.40 p.m., 18.4.31.	Remained healthy.
7. 8.	Two controls.....	{		One animal died within 1 hour and the other within 2½ hours after dosage.

DISCUSSION.

From the above table it is evident that sulphur, given to rabbits in doses of 0.5, 1.0 and 3.0 gm. daily on each of three consecutive days, prevents prussic acid poisoning when the M.L.D. of KCN per 2 Kg. rabbit is given. When twice the M.L.D. of KCN was given 0.5 gm. and 1.0 gm. of sulphur given on each of three consecutive days had very little or no effect in preventing prussic acid poisoning. On the other hand, 3 gm. of sulphur given on each of three consecutive days prevented the appearance of prussic acid poisoning even when twice the M.L.D. of KCN per 2 Kg. body weight was given.

CONCLUSIONS.

1. The white Angora rabbits employed in these experiments were much more susceptible than ordinary white short haired rabbits and dark-grey rabbits to the effects of potassium cyanide.

2. Rabbits after having received a preliminary treatment with increasing doses of potassium cyanide appear to develop a tolerance to this poison.

3. The same dose of potassium cyanide may at different times affect the same animal to a different extent.

4. Rabbits even at the point of death made a strikingly quick recovery.

5. Sulphur could be used as a successful preventive of prussic acid poisoning. Its action in this respect will largely depend on the amount of sulphur and on the dose of prussic acid given.

LITERATURE.

- HESS, W. P. (1924). Das Verhalten der Tauben unter chronischen Blausäurevergiftung. *Arch. f. Expt. Path. u. Pharm.* No. 103, p. 369.
- STEYN, D. G. (1929). Recent Investigations into the Toxicity of Known and Unknown Poisonous Plants in the Union of South Africa. *15th Rept. Dir. Vety. Serv. U. of S.A.*, Pt. 2, pp. 783-785.

Crotalariosis in Sheep.

By D. G. STEYN, B.Sc., Dr.Med.Vet., Research Officer, Onderstepoort, and

G. DE KOCK, Dr.Med.Vet., D.Sc., Deputy Director of Veterinary Services.

IN view of the interesting results obtained by Theiler (1918) by feeding *Crotalaria dura* and *C. globifera* to horses, it was decided to investigate the effects of the former plant on sheep.

The plant obtained from the Allerton Research Laboratory, Pietermaritzburg, was in a dry state and in the late seeding stage. A mixture of the stems, leaves and seeds were drenched to the sheep by means of a stomach-tube.

In the following table a summary is given of the various experiments conducted, namely the number of sheep drenched, the quantity of plant received, period between the commencement of feeding and the appearance of first symptoms, characteristic symptoms and post-mortem changes, period between the commencement of feeding and the death of the animal.

TABLE SHOWING THE EFFECTS OF DRENCHING *CROTALARIA DURA* TO SHEEP.

Sheep No.	Quantity of Plant Received.	Period from beginning of Feeding Experiment to the Appearance of First Symptoms.	Result.	Period that Elapsed from beginning of Experiment to Death of Animal.
29511	20.1.31-4.5.31 ÷ 100 grams daily * = 9,000 grams 5.5.31-3.6.31 ÷ 200 grams daily = 5,000 grams	7 days.....	There was a definite rise in temperature from 28.1.31 to 2.2.31. During this period the pulse and respiration were accelerated. The animal appeared normal until 8.5.31, when fever set in accompanied by accelerated breathing and an accelerated but strong pulse. From 10.5.31 groaning and cyanosis of the visible mucous membranes were pronounced. The respiration became progressively laboured and the pulse weaker and more accelerated until death in the night of the 3.6.31. About twelve hours before death the temperature was 105.6° <i>Post-mortem appearances:</i> Blood intensely dark reddish and tarry in consistence; subcutaneous blood-vessels distended; pronounced general cyanosis; slight ascites; hyperaemia, oedema and emphysema of the lungs with pronounced haemorrhage into the submucous tissue of the bronchi and trachea; marked emphysema of the peritracheal tissues, especially at the point of bifurcation; slight cirrhosis and degenerative changes in the liver; hyperaemia of the intestinal mucosa	135 days (specimen No. 11620).

* Except Sundays.

TABLE SHOWING THE EFFECTS OF DRENCHING *CROTALARIA DURA* TO SHEEP—
(continued).

Sheep No.	Quantity of Plant Received.	Period from beginning of Feeding Experiment to the Appearance of First Symptoms.	Result.	Period that Elapsed from beginning of Experiment to Death of Animal.
29512	20.1.31–4.4.31 ÷ 200 grams daily=13,000 grams	7 days....	<p>A temperature reaction accompanied by accelerated respiration and pulse occurred from 28.1.31 to 31.1.31. The animal appeared normal until 6.4.31, when it showed no appetite, cyanosis, accelerated pulse and respiration and was groaning. On 7.4.31 a large swelling appeared under the jaw. On palpation this swelling, which in the course of the next few days had extended down the ventral part of the neck, contained gas. The condition grew worse and death occurred in the night of 11.4.31. The pulse became progressively weaker and more accelerated and bloating appeared three days before death</p> <p><i>Post-mortem appearances:</i> Pronounced emphysema of subcutaneous tissues under jaw and on the ventral portion of the neck, extending down to the sternum; intense general cyanosis; hydropertoneum, hydropericardium; numerous subepicardial haemorrhages; marked hyperaemia, emphysema and oedema of lungs, which had a gritty feeling and on section showed numerous greyish foci; numerous haemorrhages in mucosa of trachea and bronchi; emphysema of peritracheal tissues and mediastinum hyperaemia and degenerative changes in liver; numerous subcapsular haemorrhages in spleen; acute catarrhal enteritis</p>	81 days (specimen No. 11441).
29513	20.1.31–20.2.31 ÷ 300 grams daily=8,400 grams	6 days....	<p>A temperature reaction, which persisted until death, commenced on 26.1.31. Laboured respiration, groaning, general cyanosis, apathy and inappetence continued until death. The pulse became very accelerated and weak. Death occurred in the night of the 27.2.31</p> <p><i>Post-mortem appearances:</i> General icterus; intense general cyanosis; haemorrhages in subcutaneous tissues; ascites, hydropericardium; pronounced oedema and hyperaemia of the lungs; broncho-pneumonia; hyperaemia and pigmentation and degeneration of the liver; tumor splenis; pronounced impaction of all four stomachs, the caecum and colon, acute catarrhal duodenitis and jejunitis; oedema of the retropharyngeal, mediastinal, bronchial and periportal lymph glands; degeneration and pigmentation of the kidneys</p>	38 days (specimen No. 11302).

TABLE SHOWING THE EFFECTS OF DRENCHING *CROTALARIA DURA* TO SHEEP—
(continued.)

Sheep No.	Quantity of Plant Received.	Period from beginning of Feeding Experiment to the Appearance of First Symptoms.	Result.	Period that Elapsed from beginning of Experiment to Death of Animal.
23498	16.3.31-29.4.31 ÷ 300 grams daily = 11,400 grams	12 hours...	<p>Within 12 hours after dosage the animal showed a slight rise in temperature, which with the exception of short intervals persisted throughout the course of the disease. The symptoms very closely resembled those exhibited by sheep 29513. Death occurred in the night of 29.4.31</p> <p><i>Post-mortem appearances:</i> Intense general cyanosis; venous hyperaemia of the subcutaneous blood-vessels; emphysema of the peritracheal tissues; haemorrhages into the lumen of trachea and bronchi; haemorrhage of the retropharyngeal, mediastinal and bronchial lymph glands; degenerative changes in the liver; hyperaemia of the intestinal mucosa</p>	44 days (specimen No. 11496).
29510	2.2.31-4.3.31 ÷ 400 grams daily = 10,800 grams	2 days.....	<p>Within two days after the first dose a temperature reaction, which increased towards the end of the disease, set in and continued until death. The symptoms bore a marked resemblance to those shown by the above sheep. The animal died on 5.3.31</p> <p><i>Post-mortem appearances:</i> General cyanosis; adhesive suppurating pleuritis in right thoracic cavity; catarrhal pneumonia localised in right anterior lobe; hyperaemia and oedema of the lungs; degeneration and pigmentation of the liver and kidneys</p>	30 days (specimen No. 11315).
29514	20.1.31-29.1.31 ÷ 400 grams daily = 3,600 grams	1 day.....	<p>Within 24 hours after dosage a temperature reaction set in and continued until death on 30.1.31. The train of symptoms exhibited by this animal closely resembled those in the rest of the sheep in this <i>Crotalaria</i> experiment</p> <p><i>Post-mortem appearances.</i> Slight general icterus; extensive oedema of the subcutaneous and perirectal tissues; ascites; hydrothorax; hydropericardium; subepicardial haemorrhages; hyperaemia and pneumonia of the lungs; extensive haemorrhage in peritracheal and perirenal tissues; haemorrhage in subserosal tissues of rumen; oedema of wall of gall bladder; pronounced hyperaemia; degeneration and slight cirrhosis of and haemorrhages in the liver; tumor splenis; haemorrhage into the rectum</p>	10 days (specimen No. 11230).

DISCUSSION.

Six sheep were utilized in this experiment and received daily doses ranging from 100 to 400 grams of the dry plant in the late seeding stage.

PERIOD OF LATENCY.

It is preferred to term the period which elapses from the first dose of a poisonous substance to the appearance of the first symptoms, the *period of latency*. It is felt that *incubation* period should be restricted to infectious diseases. In the past "incubation period" has almost invariably been used to designate the period which elapses from the time of infection to the appearance of the first symptoms, that is, the period which is necessary for the infection (bacteria, viruses) to propagate in the body, liberating such amounts of "toxin" as would cause disease. It appears to be quite a different matter in the case of poisons of exogenous origin and not capable of multiplying in the body.

It is evident from the foregoing table that the smaller the daily dose of the plant the longer the "period of latency", which varied from seven days in the case of a daily dose of 100 grams to one day in the case of a daily dose of 400 grams. In the case of sheep 23498 it will be noticed that a temperature reaction commenced within twelve hours of administration of the initial dose.

DURATION OF THE DISEASE.

The period from the appearance of the first symptoms up to the time of death depended, as in the case of the "period of latency", on the rate at which the plant was administered. The duration of the disease varied from 128 days in the case of a daily dose of 100 grams to 9 days in the case of a daily dose of 400 grams.

SYMPTOMS.

These resembled to a striking extent those described in horses by Theiler. The first symptoms noticed and which appeared at varying intervals after the commencement of the experiment, depending on the size of the daily dose, were fever, an accelerated but strong pulse, and increased respiration.

The course and height of the fever depended on the size of the daily dose. The 100 gram daily dose produced a one day fever of 105° F. and then 105.6° F. about twelve hours prior to death, whereas the 400 gram daily dose produced a continuous temperature, which in one case reached 106.8° F. Sheep 29511 (100 grams daily), 29512 (200 grams daily), and 23498 (300 grams daily) showed intermittent fevers, whereas sheep 29513 (300 grams daily), 29510 (400 grams daily), and 29514 (400 grams daily), exhibited continuous fevers up to the time of death.

The pulse, which became accelerated as soon as fever appeared, became progressively weaker and accelerated as death drew nearer.

The respiration likewise became progressively laboured, being more of the abdominal type.

THE PATHOLOGY OF CROTALARIOSIS IN SHEEP.

This closely resembles Theiler's (1919) classical description of Crotalariosis of equines.

The most characteristic change in the subcutis was the presence of emphysema extending from the base of the neck along the ventral cervical region to the intermandibular space. Vesicular and interstitial emphysema also occurred in the lung, and in the latter instance extended to the mediastinum along the peri-bronchial and peritracheal connective tissues.

The lungs presented a variegated appearance as a result of the oedema, hyperaemia and different stages of hepatisation. On section, in places the lung appeared flesh-like, whereas in others it was spongy as a result of the emphysema, which was so characteristic of this disease.

Microscopically the consolidated portions varied in the acute case (sheep 29514) from a red hepatisation in which the exudate was mainly serous-catarrhal and only slightly fibrinous—to a grey hepatisation. The more chronic cases (sheep 29510, etc.) revealed an irregular productive desquamated pneumonia. This was characterised here and there by clusters of epithelial cells presenting a blackish appearance, the so-called "blotches" described by Theiler. The chronic lesions presented an irregularly arranged increase of collagen fibres and fibroblastic tissue, so much so that the outlines of the alveoli could not easily be identified (c.f. sheep 29511). In this newly-formed fibroblastic matrix proliferating epithelial cells could be identified, forming irregular horseshoe-shaped or acini-like structures, whose epithelium varied from cubical to cylindrical.

Epithelial proliferations evidenced in the lungs of sheep as a result of a number of diseases was referred to by de Kock (1929). He was of the opinion that the proliferations in the lungs of sheep affected with Jaagsiekte and in the lungs of mice as described by Tyzzer (1907) and others, were of the nature of a *neoplasm*. Both these conditions are regarded as papilliform cystadenomata. On the other hand the proliferations encountered in verminous pneumonia of sheep as described by McFadyean (1920), and in the Crotalariosis in sheep referred to above, were of an entirely different nature and associated with a desquamative productive pneumonia. This will be more fully referred to by De Kock in a subsequent paper.

Besides these characteristic lung lesions of Crotalariosis referred to above, some of the sheep, furthermore, revealed a hydropericardium, ascites, venous hyperaemia of the subcutis, general icterus, multiple bronchial and tracheal haemorrhages, slight acute catarrhal enteritis, etc.

In sheep the main lesions in the *liver* were of the nature of a venous hyperaemia, which in some cases caused atrophy of the columns of liver cells. Fatty changes were present in the majority of the cases, whereas in a few instances a slight cirrhosis could also be identified.

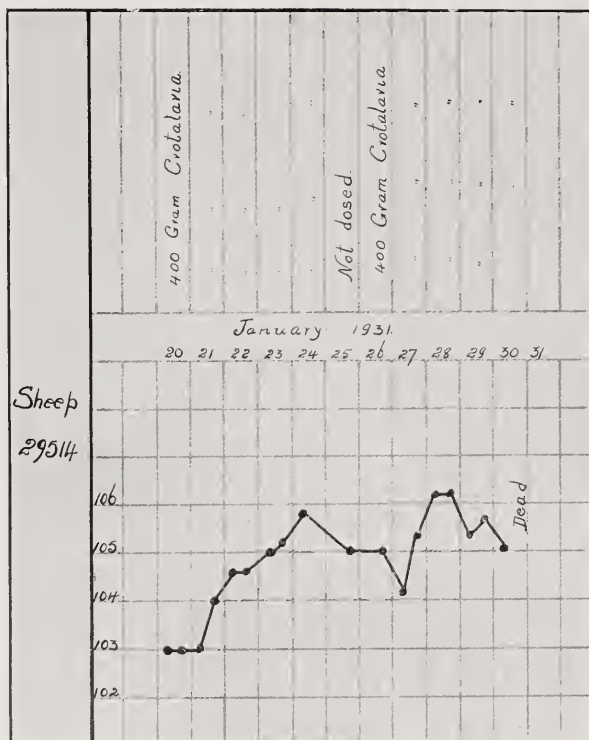
These results in *sheep* are all the more striking because Theiler maintained that the feeding of *Crotalaria dura* did not produce the lesions of a desquamative and productive pneumonia in *cattle*, but lesions in the liver of the nature of a cirrhosis and a parenchymatous hepatitis.

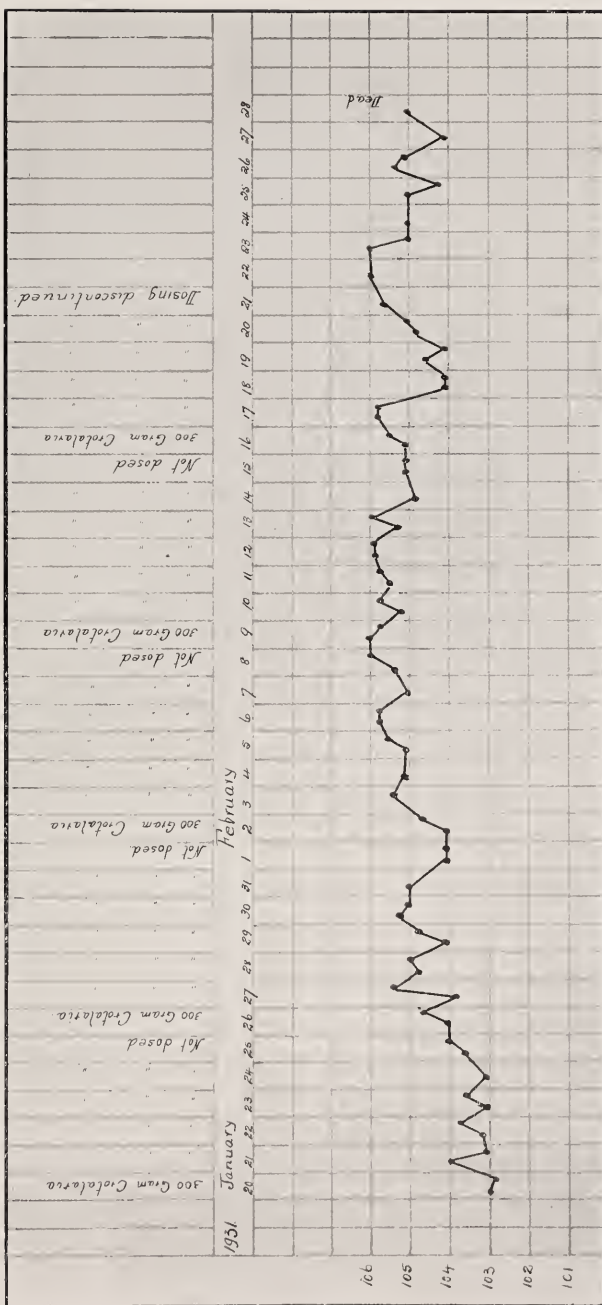
CONCLUSIONS.

It was possible by drenching to produce in sheep a *Crotalariosis* which resembled the *Crotalariosis equorum* described by Theiler

LITERATURE.

- DE KOCK, G. (1929). Are the Lesions of Jaagsiekte in Sheep of the Nature of a Neoplasm? *Annual Report of the Dir. Vet. Serv., 15th Report, 1929*, pp. 611-692.
- McFADYEAN, J. (1920). Transformations of the Alveolar Epithelium in Verminous Pneumonia in Sheep. *Jnl. of Comp. Path. and Ther.*, Vol. 33, No. 1, pp. 1-10.
- THEILER, A. (1918). Jaagsiekte in Horses (*Crotalariosis equorum*). 7th and 8th Repts., *Dir. of Vet. Res., U. of S.A.*
- TYZZER, E. E. (1907). A Series of Twenty Spontaneous Tumours of Mice. *Jnl. of Med. Res.*, Vol. 17, pp. 155-197.





Sheep 29513.

Isolation and Chemical Examination of the Poisonous Principles of *Dimorphotheca spectabilis* Schltr. and *Dimorphotheca Zeyheri* Sond.

By CLAUDE RIMINGTON, M.A., Ph.D., B.Sc., A.I.C., Research Fellow under Empire Marketing Board.

STEYN (1929) has shown by drenching experiments that the plant *Dimorphotheca spectabilis*, a member of the compositae, is lethal to sheep in doses of 100 to 130 gm. of fresh plant, the animals exhibiting all the symptoms of hydrocyanic acid poisoning. Guinea-pigs, rabbits, cattle and horses were also killed by its ingestion, the M.L.D. for the horse being approximately 1,200 gm. He was also able to demonstrate the evolution of hydrocyanic acid when the fresh plants were minced and concludes that it is probably present in the leaves in the form of a very unstable cyanogenetic glucoside since he was unsuccessful in his attempts to isolate it by any of the usual methods. *Dimorphotheca Zeyheri* also contains hydrocyanic acid and is similar in its action to *Dimorphotheca spectabilis*, the M.L.D. for sheep being, according to Steyn, approximately 150 gm.

In both species, toxicity was found to be greatest in the pre-flowering stage. At no time of the year, however, did the plants lose their toxicity completely. The plants are abundant in certain parts of the Union of South Africa and cause, yearly, fairly heavy losses of stock.

**" DIMORPHOTHECA SPECTABILIS " SCHLTR. SYNONYM:
" BIETOU " (ONDERSTEPSPOORT HERBARIUM No. A.G.
15.12.31).**

COLLECTION OF MATERIAL.

The material used in this study was collected near to the railway line from Pretoria to Johannesburg (Germiston road) at about 10 a.m. on December 14th, 1931. The plants were in the early pre-flowering stage and when bruised emitted a strong odour of hydrocyanic acid. A typical specimen was pressed for reference. A further small quantity was submitted immediately (within three hours of gathering) to the procedure described below for the determination of the " free " and " combined " hydrogen cyanide, whilst the remainder of the material was spread out in the sun to dry, a weighed sample of 50 gm. being kept apart for determination of the loss in weight on drying. When thoroughly desiccated the plants were ground to a fine powder in a pulverising mill. Feeding tests,

in order to determine the M.L.D., were carried out with this powder, using rabbits as test animals, and the "free" and "combined" hydrogen cyanide was again determined.

BOTANICAL DESCRIPTION.

The plants collected corresponded closely with the description of the species given by Phillips (1926), which is reproduced herewith:—

"A herbaceous plant 20 to 40 cm. high, with the stems arising from an underground rootstock. Stems faintly furrowed, covered with short glandular hairs. Leaves 2 to 4 cm. long, 0.6 to 1 cm. broad, lanceolate or lanceolate-ovate, tapering upwards, punctate-glandular on both surfaces, with glandular hairs beneath, ciliate with glandular hairs. Flower-heads solitary at the end of peduncles, 6 to 10 cm. long. Involucral bracts in 2 rows, somewhat connate at the base. Disc florets many, with dark tips, rays mauve-coloured. Achenes flattened, orbicular, with a circular wing. Pappus none. Recorded from the Pretoria, Witwatersrand, Heidelberg and Barberton Districts of the Transvaal."

When gathered the plants were in the fresh, unwilted condition.

DETERMINATION OF THE "FREE" AND THE "BOUND"

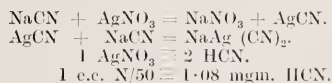
HYDROCYANIC ACID.

By "free" hydrocyanic acid is understood that quantity obtainable from a sample of the plant material under conditions such that enzymic decomposition of the contained glucoside cannot take place; it represents what is retained of the breakdown products previous to the stabilisation of the material. "Bound" hydrocyanic acid refers to that which is still in combination as glucoside and is calculated from the difference between the "free" and total hydrocyanic acid, the latter being determined after allowing enzymic hydrolysis to proceed to completion.

The method finally adopted for hydrogen cyanide determination is based upon the standard procedure due to Liebig for the determination of soluble cyanides by silver nitrate in an alkaline medium. Its accuracy was checked by use of standard sodium cyanide solutions and also by the application of a titration method based upon the reaction between copper solutions and soluble cyanides.

A suitable quantity of the plant material, yielding about 5 to 10 mgm. of hydrogen cyanide, is dropped into 20 c.c. of water, nearly at boiling point, in a 500 c.c. flask. A little sand is then added to diminish foaming and 10 gm. of tartaric acid and the flask immediately closed with a rubber stopper carrying a steam inlet tube passing to the bottom of the flask, and a splash-bulb outlet connected to a water-cooled Liebig's condenser, the adapter of which dips below the surface of a solution of sodium hydroxide (5 c.c. normal alkali plus about 10 c.c. of water) contained in a small flask. A brisk current of steam is now passed through the apparatus until approximately 150 c.c. of liquid have distilled over into the receiving flask. To avoid condensation of the steam in the 500 c.c. flask, this is heated so that the contents are maintained at the boiling point. The

distillate together with the washings of the condenser is now titrated by 1/50 normal silver nitrate solution, a crystal of potassium iodide being added to render the end-point sharper and a few drops of toluene which effectively removes substances having a tendency to produce turbidity. Since the silver cyanide at first formed redissolves as long as free alkali cyanide is present, the end-point is determined by the appearance of a permanent white turbidity, when exactly one-half equivalent of silver nitrate has been added. 1 c.c. of N/50 AgNO_3 thus represents 1.08 mgm. HCN.



In determining the total hydrogen cyanide, from which that "bound" is calculated by subtraction, the procedure employed is as follows. A suitable quantity of the plant material is introduced into 200 c.c. of a citrate buffer mixture having pH 6.0 and contained in a tightly stoppered 500 c.c. flask. After maceration for 24 hours at room temperature, 10 gm. of tartaric acid and some sand are introduced, the flask being quickly attached to the steam distillation apparatus and the determination then carried out as described above for the case of the free hydrogen cyanide. The citrate buffer is prepared as follows:—

Solution A.—21.008 gm. crystallised citric acid together with 200 c.c. of N NaOH in sufficient water to make 1 litre.

Solution B.—N/10 NaOH.

For stock solution, mix 241 c.c. of solution A and 156 c.c. of solution B. 200 c.c. is sufficient for about 10 gm. (dry weight) of plant material.

The method is simple, dependable and rapid, and is to be preferred to the colorimetric procedure of Viebover and Johns (1915). Titration of the alkaline distillate, to which about 1 gm. of solid ammonium sulphate had been added in order to liberate ammonia, with N/25 copper sulphate solution until a faint blue colour persisted gave results in agreement with those determined by silver titration. It was found advisable, however, to steam the apparatus out for two or three hours when first set up since new rubber stoppers and tubing liberate small quantities of hydrogen sulphide which, reacting with the silver, renders the solution slightly brown and turbid, thereby greatly diminishing the sensitivity of the eye in judging the end-point. It is advisable not to add chloroform or toluene as a preservative during the period that the plant material is macerating since these substances tend to inhibit the enzyme action (Brünnich 1903). Occasionally plants are encountered which contain some substance capable of combining with hydrogen cyanide, the quantity of which steadily diminishes as maceration proceeds. (Alsberg and Black, 1916.) In the case of the *Dimorphotheca* species, however, this did not happen.

Results: *Dimorphotheca spectabilis*; fresh plant cut up by scissors (moisture content 77.6 per cent.).

Free HCN: 0.052 gm. HCN/100 gm. dry weight.
Total HCN: 1.690 gm. HCN/100 gm. dry weight.

It is evident that the plant must contain an exceedingly large quantity of cyanogenetic glucoside.

The distribution was as follows (material gathered 19.3.32 in same locality):—

	Moisture Percentage.	HCN Percentage of Fresh Material	HCN Percentage on Dry Weight Basis.
Whole plant..	79.12	0.289	1.382
Green stems..	73.72	0.114	0.435
Leaves.....	83.49	0.292	1.766

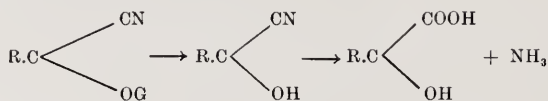
As an illustration of the great rapidity with which the glucoside is hydrolysed by the enzymes present in the plant, it may be recorded that a 50 gm. sample, after having been put rapidly through a mincing machine, yielded as much as 0.505 gm. "free" HCN/100 gm. dry weight, nearly 10 times the quantity found to be present when the material was simply cut up by scissors.

After sun-drying and grinding (9 days after the material was picked) the "free" and total HCN was again determined with the following results:—

Free HCN: 0.022 gm. HCN/100 gm. dry plant.

Total HCN: 1.061 gm. HCN/100 gm. dry plant.

There is thus an appreciable loss of glucoside owing to enzyme action whilst the plant is being dried. Further information upon this point is recorded under *Dimorphotheca Zeyheri*. Distillation of the plant material (and also of the glucoside subsequently isolated) with dilute sulphuric acid yielded only about 10 per cent. of the total quantity of hydrogen cyanide in the free form. Possibly hydrolysis to the nitrile occurs to some extent, as is also true in the case of amygdalin:—



The following data serve to illustrate this point.

An extract of 2 gm. of plant powder was made with boiling water, using 3 successive portions; total volume 70 c.c. One half was distilled with sulphuric acid, the other added to 0.5 gm. of plant powder, the mixture allowed to macerate overnight and then distilled in the usual way:—

35 c.c. extract distilled with 2.5 c.c. of 10 per cent. H_2SO_4 nearly to dryness, yielded.....	0.86	mgm. HCN.
35 c.c. extract macerated with 0.5 gm. of plant, then steam-distilled, yielded.....	16.36	..
Control 0.5, of plant macerated alone, then steam-distilled, yielded.....	5.51	..
∴ by difference, 35 c.c. of extract yielded	10.85	..
By theory (twice control).....	11.02	..
Acid liberated only 7.84 per cent. of the bound HCN.		

FEEDING TESTS.

The toxicity of the ground, dried material was determined by administering weighed quantities, mixed with a little water, to rabbits by stomach tube. It was found that, whereas 2.0 gm. were without effect, 2.5 gm. given to a two-kilogram rabbit was sufficient to cause death from hydrocyanic acid poisoning within about twenty minutes. (See Appendix.)

The M.L.D. lies, therefore, between 1.0 and 1.25 gm. per kilo body weight for the rabbit, a figure which corresponds to about 11 mgm. of hydrogen cyanide. The lethal dose of sodium cyanide for the rabbit corresponds to about 5 to 6 mgm. of hydrogen cyanide per kilo, hence it appears that the toxicity of *Dimorphotheca* is wholly accountable by the hydrogen cyanide it is capable of liberating.

PROXIMATE ANALYSIS OF THE PLANT.

The ground plant powder was employed and the following determinations carried out, using the official methods of the American Agricultural Society as recorded in their monograph (1930). In view of the association noted by Treub (1905) between the quantity of cyanogenetic glucoside and of potassium nitrate in *Phaseolus lunatus*, it was considered to be of interest to determine the amount of potassium present in the ash of *Dimorphotheca spectabilis*. The quantity found* corresponded to 2.07 per cent. of the dry plant powder, an unusually high figure.

	Percentage.
Moisture content of fresh plant.....	77.6
Ash calculated on dry weight.....	8.86
Crude protein on dry weight.....	16.19

ISOLATION OF THE CYANOGENETIC GLUCOSIDE.

Two different procedures were adopted depending upon whether the initial stabilisation of the material took place with alcohol or with boiling water. Previous failures to isolate the glucoside, which is readily obtained in the crystalline condition, have almost certainly been due to the lack of precautions taken to inactivate the hydrolytic enzymes present in the plant.

1. Alcohol Extraction.

500 gm. of plant powder (HCN = 1.06 per cent.) was left for 5 hours in contact with 2 litres of 90 per cent. alcohol and a little solid calcium carbonate. The liquid was then strained off and the extraction repeated overnight with a further 2 litres of 90 per cent. alcohol. After straining, a final extraction was made with 1½ litres of boiling 90 per cent. alcohol. The combined extracts were filtered and concentrated to about 250 c.c. under reduced pressure at a temperature not exceeding 60° C. On diluting with water, much sticky, resinous material separated. This was filtered off and the deep yellow liquid treated with basic lead acetate until no more precipitate formed.

* I wish to thank Mr. J. Louw for carrying out this determination.

After filtration, the yellow liquid was treated with sulphuric acid to remove most of the lead as sulphate, after which it was neutralized and again concentrated firstly by vacuum distillation and finally in the serum drier to the consistency of a syrup. This was dissolved in 300 c.c. of alcohol and an equal volume of ether added. A thick, yellow oil precipitated which was removed and the clear liquid again evaporated to a syrup, dissolved in 50 c.c. of absolute alcohol and poured into 250 c.c. of ether. The precipitate which separated was light yellow and of a buttery consistency. It contained some glucoside but was largely composed of inorganic material and reducing sugars. The alcohol-ether solution was allowed to evaporate, leaving a mass of fine rosettes of exceedingly slender needles together with some resinous material. It was scraped into centrifuge cups, well

Fig. 1.



Glucoside from *Dimorphothecca spectabilis*,
crystallised from Alcohol. $\times 15$.

washed with ether, then dissolved in absolute alcohol and precipitated by addition of anhydrous ether several times in succession and finally crystallised from alcohol and the crystals washed with ether. As thus obtained, it was a colourless crystalline material melting at 139° (uncorr.) with decomposition. From water or alcohol it crystallised in rosettes of very slender needles (Fig. 1), on separating more slowly from absolute alcohol, in crusts of needles, and from ethyl acetate in fine silky needles. It is very readily soluble in water and alcohol, moderately soluble in ethyl acetate and aniline, insoluble in petroleum ether and ether. It does not reduce Fehling's solution until after hydrolysis. Upon distilling with acid, hydrogen

cyanide is formed and a volatile substance giving the iodoform reaction and Rothera's test for acetone. With concentrated sulphuric acid it gives no colour reaction, but when sulphuric acid is added to an aqueous solution of the glucoside, in which a crystal of rhamnose has been dissolved, a fine crimson ring is seen at the junction of the two liquids (reaction between methylfurfural and acetone).

Elementary micro-analysis [§] afforded the following figures:—

Moisture content : 0.1014 gm. crystalline glucoside was dried at 104° for 3½ hours.			
Loss of weight : 0.0008 gm.; the substance crystallises, therefore, uncombined with solvent.			
Found	C	H	N
C ₁₀ H ₁₇ O ₆ N	48.53	6.95	5.58
requires	48.54	6.93	5.67

The specific rotatory power was determined in aqueous solution against sodium light, using a Goerz triple-field instrument:—

0.2536 gm. glucoside dissolved in 13 c.c. of water gave a rotation of -1.06° in a 2 dm. tube at 27° C.

$$\begin{aligned} \text{Hence } [\alpha]_D^{27} &= \frac{-1.06 \times 13 \times 100}{2 \times 25.36} \\ &= -27.17 \end{aligned}$$

In all the above details the glucoside isolated from *Dimorphothea spectabilis* resembles closely the cyanogenetic glucoside "linamarin" or "phaseolunatin" which was first isolated by Jorissen and Haïrs (1891) from flax. It was later obtained by Dunston, Henry and Auld (1906A) from flax and from *Manihot aipi* and *utilissima* (1906B), by Dunston and Henry (1904) from "Lima beans", *Phaseolus lunatus*, and by Rosenthaler (1922A) from *Dimorphothea ecklonis*. Phaseolunatin has also been synthesised by Fischer and Anger (1919) starting with β -acetobromoglucose and ethylhydroxyisobutyrate. That the glucoside of *Dimorphothea spectabilis* and "phaseolunatin" are identical is shown by a comparison of their physical properties.

	Cyanogenetic glucoside from <i>Dimorphothea spectabilis</i> .	'Linamarin' from <i>Linum usitatissimum</i> (Jorissen and Haïrs).	'Linamarin' from <i>Linum usitatissimum</i> (Dunston, Henry and Auld).	'Phaseolunatin' from <i>Phaseolus lunatus</i> (Dunston and Henry).	'Phaseolunatin' from <i>Manihot</i> species. (Dunston, Henry and Auld).	'Linamarin' from <i>Dimorphothea ecklonis</i> . (Rosenthaler).
Melting point....	139° — 27.2°	134°	138° — 27.4°	141° — 26.2°	138°	144° — 28.65°
$[\alpha]_D^{27}$	Rosettes of slender needles; also larger prisms (β form)	'Colourless needles'	'Spreading rosettes of slender needles'	'Rosettes of colourless needles'	'Spreading rosettes of colourless needles'	'Whitesilky needles'
Crystalline form, ..						
Elementary composition— C ₁₀ H ₁₇ O ₆ N requires—						
C	48.54%..	C 47.9%..	C 48.1%..	C 48.19%..	—	—
H	6.93%..	H 6.95%..	H 6.8%..	H 6.91%..	—	—
N	5.67%..	N 5.58%..	N 5.6%..	—	—	—
Action of emulsin	—	—	—	—	—	—
H ₂ CN contained in the plant.....	1.690% in whole plant	—	0.13% in whole plant	0.10% in dark beans	0.035% in dried root of root	1.217% in leaves; 0.374% in in stems

For the synthetic glucoside, Fischer and Anger (1919) give M.P. 141–2°, $[\alpha]_D^{18} = -29.1^\circ$.

[§] Analysis by Dept. of Organic Chemistry, University of the Witwatersrand 961

IDENTIFICATION OF THE DECOMPOSITION PRODUCTS.

Hydrogen cyanide was identified by (1) its action on picrate paper, (2) the formation of prussian blue and of silver cyanide in the distillate after enzymic or acid hydrolysis. For quantitative determination, 65 mgm. of glucoside was dissolved in buffer solution, to this 0.25 gm. of plant powder was added and, after maceration overnight at room temperature, the liberated hydrogen cyanide was determined by steam distillation in the usual way. Another portion of 0.25 gm. of plant in buffer solution was treated similarly as a control. The results obtained were as follows:—

65 mgm. glucoside + 0.25 gm. plant yielded.....	9.180 mgm. HCN.
Control 0.25 gm. plant yielded.....	2.646 „
∴ 65 mgm. glucoside yielded.....	6.534 „

$$\text{Theory requires } \frac{27}{247} \times 65 = 7.10 \text{ mgm. HCN.}$$

The yield was therefore 92.1 per cent. of theory.

Acetone was shown to be present in the distillate by positive iodoform and nitroprusside reactions and also by the isolation of its condensation product with benzaldehyde, dibenzylidene-acetone. 0.25 gm. of the glucoside was dissolved in water and to this was added a few mgm. of dried, powdered *Dimorphotheca spectabilis* in order to supply the hydrolytic enzyme. After standing overnight, the mixture was acidified with sulphuric acid and distilled, the distillate being condensed in a flask surrounded by melting ice. In order to remove the hydrocyanic acid from this solution, freshly precipitated lead hydroxide was added in excess and after an hour the mixture centrifuged. The clear supernatant liquid had an acetone-like odour and gave a strongly positive iodoform reaction.

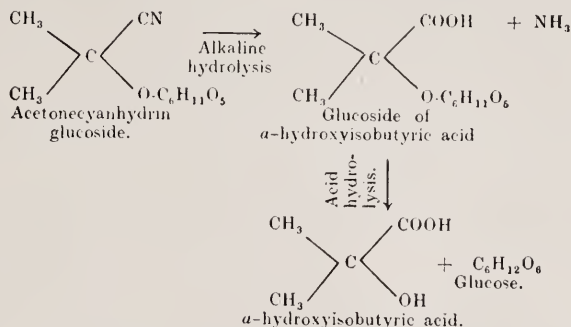
To this liquid 0.5 c.c. of benzaldehyde was added followed by 1 c.c. of N sodium hydroxide and sufficient 96 per cent. alcohol to dissolve the benzaldehyde. After standing for some hours, the mixture became cloudy and thin, glistening plates of a lemon-coloured crystalline substance were deposited. These were filtered off, collected on a porous plate, and allowed to dry. They proved to be identical with a specimen of dibenzylidene-acetone similarly prepared from 0.5 c.c. acetone dissolved in 15 c.c. of water.

Crystals isolated:	
Pale yellow plates M.P.....	106–107°
Authentic specimen of Dibenzylidene-acetone:	
Pale yellow plates M.P.....	108°
Mixed M.P.....	106°

ALKALINE HYDROLYSIS OF THE GLUCOSIDE AND ISOLATION OF α -HYDROXYISOBUTYRIC ACID.

Since the glucoside is a cyanhydrin, alkaline hydrolysis should convert it into the corresponding acid with elimination of ammonia,

most probably whilst leaving intact the glucosidic linkage. Subsequent acid hydrolysis would then remove the sugar, leaving behind the hydroxy acid corresponding to the original carbon chain:—



0.5 gm. of the glucoside was dissolved in 10 c.c. of saturated barium hydroxide solution and the mixture heated on the water bath, under reflux, for 2 hours. To the pale yellow liquid, which smelt strongly of ammonia, sufficient sulphuric acid was added to give a distinct acid reaction to litmus paper. The solution was then filtered, boiled gently, under reflux for 15 minutes, cooled and extracted with ether. The ethereal extracts were dehydrated over solid calcium chloride and the ether removed by evaporation. There remained a pale yellow oil, acid in reaction, and possessed of a characteristic, rancid odour. It was dissolved by warming in a little water, sufficient barium hydroxide solution added to give a neutral reaction and the resulting solution allowed to evaporate at the temperature of the laboratory. A deposit of minute colourless crystals, prisms or needles, was left which were similar in appearance to barium α -hydroxyisobutyrate. Analysis after drying at 105° :

$$\text{Ba} = 38.5 \text{ per cent.}$$

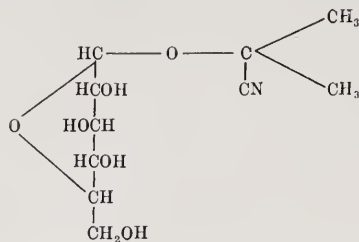
$$(\text{C}_4\text{H}_7\text{O}_3)_2\text{Ba} \text{ requires Ba} = 39.9 \text{ per cent.}$$

The aqueous liquid left after extracting the α -hydroxyisobutyric acid with ether, possessed reducing properties. In order to identify the sugar residue, it was neutralized and evaporated to a volume of about 15 c.c. To this 0.15 gm. of phenylhydrazine hydrochloride was added, a drop of glacial acetic acid, and 0.3 gm. of sodium acetate and after a few minutes the solution was filtered into a test tube which was immersed in a boiling-water bath.

The osazone which separated in the course of a few hours was filtered off, washed first with water and then once with 50 per cent. alcohol and dried on a porous plate. The crystals were in sheaf-like aggregates of fine needles and had M.P. 206° .

The osazone was therefore glucosazone, which when pure has M.P. 206° . The melting point was not lowered by admixture with an authentic specimen of glucosazone.

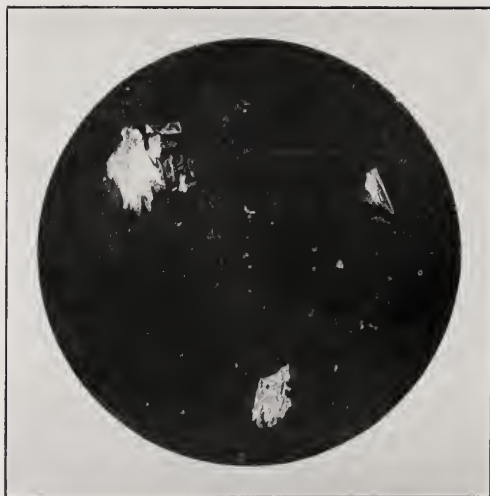
The constitution of the glucoside is therefore established as the glucose ether of acetonecyanhydrin and its structural formula may consequently be written:—



A NEW CRYSTALLINE FORM.

It was noticed that if ether was added to a rather dilute solution of the glucoside in absolute alcohol until a slight turbidity appeared, the crystals which were deposited on standing, differed in general appearance from those hitherto described as typical of the glucoside. They formed small clusters of translucent prisms, or flattened needles, which projected from the walls of the tube into the surrounding liquid (see Fig. 2), not, as is characteristic of the more usual form, spreading in rosettes upon the surface of the glass.

Fig. 2.



Glucoside from *Dimorphotheca spectabilis*
 β form crystallised from alcohol and ether. $\times 15$.

Some of these crystals which, for convenience, may be referred to as the " β form", were dried at a temperature of 95° for two hours but no loss in weight occurred. After about ten minutes at

this temperature, the whole mass was observed to become fluid and then to solidify in the characteristic rosettes of the typical form. The " β " crystals do not, therefore, contain solvent of crystallisation; it would appear that the glucoside is dimorphous, the " β " form being the less stable. Confirmation of this suggestion was afforded by the demonstration that the " β " crystals, slightly moistened with water upon a glass slide, could be converted into the typical rosette-form by scratching with a needle which had been previously soiled with the more stable form. The photograph reproduced in Fig. 3 shows very clearly the way in which the rosettes surround the scratch lines. Both forms appear to have the same melting point, or at any rate the reversion to the more stable form occurs so rapidly on heating that the change is complete before any difference in fusion temperature can be noticed.

Fig. 3.



Glucoside from *Dimorphotheca spectabilis*
 β form transformed into normal (rosette form)
 by scratching. $\times 15$.

II. Isolation of the Glucoside by water Extraction.

As an alternative method of stabilizing, the plant material can be dropped into boiling water containing a little solid calcium carbonate. 100 gm. material was extracted in this way with successive portions of 750, 750 and 500 c.c. of water. The combined extracts

were filtered, precipitated by neutral lead acetate, excess of lead removed by hydrogen sulphide and the filtrate concentrated under reduced pressure to a volume of 300 c.c. It was then neutralised and further concentrated in the serum drier until no more moisture could be removed. The volume of the syrup was about 50 c.c.; 150 c.c. of absolute alcohol were added and the clear mixture poured into 400 c.c. of alcohol which precipitated a quantity of yellow material. This was removed on the centrifuge and washed with hot alcohol, the washings and supernatant fluid being again concentrated in the serum drier. The syrup, at this stage, weighed 31.5 gm. and showed some tendency to crystallise. It was dissolved in alcohol, impurities precipitated by 2.5 volumes of ether and after filtration the addition of ether continued up to a total of 6 volumes. The last precipitate which formed was worked up again for the glucoside it contained but the major portion was found in the alcohol-ether solution from which it crystallised on concentration. Recrystallisation was effected from alcohol. The water extraction method is possibly the more convenient, although the removal of inorganic matter and other impurities at the end stages necessitates repeated fractionation from alcohol-ether mixtures, a laborious process attended by considerable losses of glucoside. In the case of *Dimorphotheca Zeyheri* a record was kept of the losses at each stage but little improvement in yield resulted.

**“ DIMORPHOTHECA ZEYHERI ” SOND. SYNONYM:
“ JAKHALSBOS”.**

Phillips (1926) gives the following description of the plant:—

“ Dwarf perennial, with annual shoots springing from an underground rootstock. Stems scaberulous. Leaves 2.5 to 6 cm. long, 0.2 to 1 cm. broad, linear to oblong, obtuse, entire, toothed or lobed, scabrous. Flower-head solitary, on a short peduncle, 4 cm. in diameter, including the rays. Disc florets many, producing flattened achenes. Ray florets yellow, producing 3-angled achenes. Pappus none. Recorded from the Prieska, Kimberley and Middelburg Districts of the Cape Province.”

The material investigated was a sample of the ground-up plant for which I am indebted to Dr. Steyn. Its origin is given as: “P.O. Meadows, Bloemfontein” with the date 10.8.28. When examined by the methods already described, a free hydrogen cyanide content of 0.045 per cent. and a total of 0.267 per cent. was found. Since Steyn (1929) found this material when freshly gathered to be nearly as toxic as *Dimorphotheca spectabilis*, it can be assumed that its glucoside content was of the same order. Hence it follows that by enzymic decomposition, while stored in the air-dried state, the hydrocyanic acid content had in three years fallen from about 1 per cent. to $\frac{1}{4}$ of this value. The distribution of hydrocyanic acid in the different parts of the plant was determined upon specimens gathered

for the purpose in the poison garden at the Laboratory (Peg specimen 136, 30.10.31). The technique followed was similar to that previously described under *Dimorphotheca spectabilis*. Results: Material gathered 21.3.32:—

	Moisture Percentage.	HCN Percentage of Fresh Material.	HCN Percentage on Dry Weight Basis.
Flowers.....	71.86	0.043	0.153
Green Stems..	69.56	0.026	0.085
Leaves.....	75.93	0.257	1.068
Proximate analysis of the dry plant powder gave ash			8.45 %
Crude protein.....			11.19 %

ISOLATION OF THE GLUCOSIDE.

Using 100 gm. of the dried powder, the water extraction process was followed. Quantitative determinations of the amount of glucoside lost in each precipitate were made by allowing these to macerate with 0.5 gm. of ground *Dimorphotheca spectabilis* followed by distillation and silver titration. A control of 0.5 gm. of *D. spectabilis* alone was set up concurrently in each case in order to determine the quantity of hydrogen cyanide coming from this source. The results tabulated below are self explanatory:—

Original total HCN/100 gm. plant.....	267.0 mgm.	
Free HCN.....	45.3 mgm., i.e.	16.96 %
First alcohol: ether precipitate (1 : 1).....	12.96 mgm., i.e.	4.86 %
Second alcohol: ether precipitate (1 : 1)....	4.32 mgm., i.e.	1.62 %
Third alcohol: ether precipitate (1 : 3).....	36.93 mgm., i.e.	13.83 %
Small residues.....	8.64 mgm., i.e.	3.24 %
Total.....		40.51 %

0.79 gm. of pure glucoside was isolated, of which the HCN equivalent is 86.38 mgm, i.e. 32.34 per cent.

∴ Unaccounted for in recrystallisation residues, etc., 27.15 per cent.

Yield calculated on bound HCN = 39 per cent.

It is clear that the heaviest losses occur in the last stages, but no way of obviating this was found. The material isolated was in every way identical with the glucoside obtained from *Dimorphotheca spectabilis*. It had M.P. 137°.

Analysis—	C	H	N
Found.....	48.84	7.05	5.83
C ₁₀ H ₁₇ O ₆ N requires.....	48.54	6.93	5.67

THE ENZYMES CONCERNED IN THE DECOMPOSITION OF THE GLUCOSIDE.

There seems to be some confusion in the literature as to whether or not "linamarin" or "phaseolunatin" is hydrolysable by the β -glucosidase emulsin. Jorissen and Hairs (1891) stated that it was not so attacked. Dunston, Henry and Auld at first came to the opposite conclusion; in a later paper, however, they (1907) describe a reinvestigation of the point leading them to assert the stability of the glucoside towards emulsin, but its ready hydrolysis by the α -glucosidase maltase, present in yeast. The sugar formed during its decomposition was considered to be α -glucose. In the present study, the behaviour of the glucoside towards the two enzymes, emulsin and maltase, was examined as follows: 2 c.c. of a dilute aqueous

solution of the glucoside was pipetted into each of two test tubes. To the first was added a few milligrams of an active emulsin, prepared from bitter almonds. A control tube containing emulsin and amygdalin was set up alongside of this. To the second glucoside tube was added 1 c.c. of an extract of dried bottom yeast prepared as described by Fischer (1894) and shown to contain an active α -glucosidase by its power to hydrolyse maltose. In the top of each stoppered tube was placed a strip of sodium picrate paper. After a few hours at room temperature, signs were noted of the liberation of hydrocyanic acid from the amygdalin. No darkening of the picrate paper occurred even after four days in the remaining tubes. Hence it can be concluded that the glucoside is not hydrolysed either by maltase or by emulsin.

THE NATURE OF THE ENZYMES PRESENT IN THE PLANT.

In a precisely similar manner to that already described, the action of a preparation of the enzyme from *Dimorphotheca spectabilis* was tested upon amygdalin, salicin, the glucoside and maltose (the latter polarimetrically). A preparation of the enzyme, free from hydrocyanic acid, was made as follows: 25 gm. of plant powder was extracted overnight at room temperature with 175 c.c. of water. The strained liquid was centrifuged, solid ammonium sulphate added to saturation and the precipitate centrifuged off and washed with saturated aqueous ammonium sulphate solution. It was then dissolved in 10 c.c. of water. This solution was found to liberate hydrocyanic acid from the *Dimorphotheca* glucoside with great rapidity, from emulsin fairly readily, but to attack salicin only slowly, the change into saligenin, which gives a purple colour with ferric chloride, being taken as evidence of hydrolysis. Upon maltose it had no detectable action in 48 hours. The results are summarized below:—

<i>Dimorphotheca</i> enzyme preparation	plus Amygdalin	Salicin	Maltose	<i>Dimorphotheca</i> glucoside.
	+	slowly	+	—
„ boiled	—	—	—	—
				++

It would appear that the plant contains an enzyme capable of decomposing the glucoside with liberation of hydrogen cyanide but identical with neither emulsin (β -glucosidase) nor with maltase (α -glucosidase). The action upon amygdalin, a cyanogenetic β -glucoside, is fairly rapid; but upon salicin, the β -glucoside of saligenin, it is only just detectable.

In the case of *Dimorphotheca ecklonis*, Rosenthaler (1922 b) concluded that the glucosidase of that plant was a specific enzyme distinct from either emulsin or maltase. Following the usual custom, the term “Linamarase” may be retained in this case also. The enzymes present in *Phascolus lunatus*, *Manihot* species, etc., possess a wider range of activity.

TOXICITY OF THE GLUCOSIDE.

The poisonous action of plants containing cyanogenetic glucosides is due to the hydrogen cyanide liberated by the action of the appropriate enzymes they contain. Apart from its enzyme, the

glucoside is usually stable and non-toxic. In feeding experiments carried out to ascertain the toxicity of the *Dimorphotheca* glucoside, controls were carried out with an amygdalin preparation (Merek), a cyanogenetic β -glucoside typical of its class. Alone it had no effect in doses as large as 0.5 gm. (given to a 1,900 gm. rabbit by stomach tube) corresponding to approximately 30 mgm. of hydrogen cyanide, nearly three times the normal lethal dose. Even when a very active emulsin preparation was simultaneously administered, this quantity of glucoside was insufficient to produce death. (See Appendix.)

It must be remembered that the optimal acidity for the action of emulsin lies at about pH 6.0; in contact with the highly acidic gastric contents its glucosidolytic activity would be almost entirely suppressed. The conditions obtaining when the fresh plant or plant powder is ingested differ from experiments with the isolated glucoside in one fundamental respect, both substrate and enzyme are still enclosed within the plant cell, the proteins, etc., of which constitute a very efficient buffering system, hence interaction may take place at a pH approaching the optimal and rapid liberation of hydrogen cyanide from the glucoside occur before the mass becomes permeated with the acid gastric contents. Just as is the case with amygdalin, it was found impossible to produce symptoms of poisoning when even as much as 0.1 gm. of the *Dimorphotheca* glucoside followed by 0.25 gm. of dry plant powder (to supply the enzyme) was fed to rabbits of 1,700 to 2,000 gm. weight. By allowing the enzymic decomposition of the glucoside to take place prior to administration, the results were, however, regularly positive with doses of about 90 mg. of glucoside, equivalent to 1 M.L.D. in terms of hydrogen cyanide. For example, to 90 mg. of glucoside dissolved in 10 c.c. of buffer solution pH 6.0, about 1 mg. of plant powder (*D. spectabilis*) was added and the mixture left in a tightly stoppered flask overnight. A piece of picrate paper showed that liberation of hydrogen cyanide was taking place. The mixture was administered by stomach-tube to a rabbit weighing 1,700 gm. Within 2 minutes after dosing, the respiration became very laboured and a general weakness was observed; the head then sank to one side, whilst the respiratory distress became more acute. After passing into coma, the animal died 7 minutes after dosing. The post-mortem examination showed all the typical signs of hydrogen cyanide poisoning. As a control, 2 gm. of plant powder alone was administered to another animal, but no ill effect supervened. As previously stated, the M.L.D. of the powder was found to be between 2 and 2.5 gm. for a 2-kilo rabbit.

PROPHYLAXIS AND TREATMENT.

Steyn (1929) has been able to demonstrate the beneficial action of sulphur administered to animals as a prophylactic and also simultaneously with an otherwise lethal dose of *Dimorphotheca spectabilis*. As a preventive it is suggested that sulphur be administered in the form of a lick (it can be incorporated with one or other of the different kinds of lick generally in use), 0.5 lb. per 100 sheep, since the dose has to be renewed every second day for complete protection. Most probably the hydrocyanic acid is detoxicated by conversion into the non-poisonous sulphocyanide.

The method, which is cheap and simple, is said to give excellent results in practice.

I wish to acknowledge my indebtedness to Dr. D. G. Steyn for his assistance in the collection and preparation of the material used in these studies, and for kindly placing his records at my disposal; also for his help in making the post-mortem examinations.

SUMMARY.

The toxic substance present in the plants *Dimorphotheca spectabilis* Schltr. and *Dimorphotheca Zeyheri* Sond. has been isolated in pure crystalline condition and identified. Both plants contain the same principle. It is a cyanogenetic glucoside, identical with "linamarin" or "phaseolunatin", and is thus the glucose ether of acetonecyanhydrin. A hitherto unreported crystalline form is described; the glucoside is therefore dimorphous.

The enzymes emulsin and maltase have little or no action upon the glucoside; it is readily hydrolysed, however, by an enzyme present in the plants. The glucosidase present in *Dimorphotheca spectabilis* and *Zeyheri* has only a relatively feeble action upon amygdalin and is unable to hydrolyse maltose. It is not identical, therefore, with either emulsin or maltase and may be provisionally referred to as "linamarase" in order to indicate its selective action upon the glucoside. Quantitative determination, by a method which is described, of the hydrogen cyanide liberated by enzymic decomposition shows that in the fresh state *Dimorphotheca spectabilis* is capable of yielding about 1.7 gm. HCN and *Dimorphotheca Zeyheri* about 1.1 gm. HCN per 100 gm. plant (dry weight basis).

APPENDIX.

Feeding tests carried out with Dimorphotheca spectabilis and Zeyheri and products derived therefrom.

I. Determination of the M.L.D. of the dried plant powder.

Rabbit No. 1, weight 2,660 gm. received 2.0 gm. *D. spectabilis* powder in 40 c.c. of water by stomach-tube at 12.15 p.m. on 4.1.32.

By 4.30 p.m. no symptoms had developed.

Rabbit No. 2, weight 2,700 gm. received 5.0 gm. of same powder in 50 c.c. water by stomach-tube at 12.25 p.m. on 4.1.32. 12.28 p.m. head falls to one side, respiration slow and laboured; 12.35 p.m. gasping, very weak, lying on side; 12.40 p.m. died.

Post-mortem appearances: General cyanosis, marked hyperaemia of lungs, liver and kidneys, pronounced dilatation of both ventricles of the heart; slight reddening of the mucous membrane of the stomach.

Conclusion: Typical hydrocyanic acid poisoning.

Rabbit No. 3, weight 2,500 gm. received 2.5 gm. plant powder in water by stomach-tube at 9.25 a.m. on 5.1.32. 9.35 a.m. on side, breathing hard; 9.40 a.m. respiratory distress acute, breathing in gasps; 9.42 a.m. slight convulsion followed by death.

Post-mortem appearances: General cyanosis; hyperaemia of lungs, liver and spleen; pronounced dilatation of both ventricles of the heart; stomach distended; odour of hydrogen cyanide when incised.

Conclusion: Typical hydrocyanic acid poisoning.

Rabbit No. 4, weight 2,300 gm. received 2.25 gm. plant powder in water by stomach-tube at 9.36 a.m. on 6.1.32. 9.43 a.m. restless, breathing heavily with mouth open. Salivation; 9.51 a.m. falls on one side with head retracted; 9.56 a.m. comatose, gasping; 10.01 a.m. died.

Post-mortem appearances: General cyanosis; hyperaemia of lungs and liver; spleen normal; both ventricles of the heart dilated; stomach distended with slight haemorrhagic spots visible.

Conclusion: Typical hydrocyanic acid poisoning.

General Conclusion: The M.L.D. of the plant powder lies between 2 and 2.25 gm. for a rabbit of approximately $2\frac{1}{4}$ Kilograms.

II. Tests carried out with amygdalin.

Rabbit No. 5, weight 1,700 gm. received 0.05 gm. amygdalin, dissolved in water, by stomach tube.

As no symptoms appeared within 10 minutes, this was followed by 0.05 gm. of emulsin dissolved in water and administered by the same route. No symptoms within 15 minutes.

A further dose of 0.5 gm. of amygdalin followed by 0.05 gm. of emulsin was then given. No symptoms within 1 hour.

Conclusion: The cyanogenetic glucoside, given alone or together with the enzyme which decomposes it, is not toxic to the rabbit even when administered in a dose corresponding to 3 M.L.D. on the basis of the hydrogen cyanide it is capable of liberating.

III. Tests with the glucoside isolated from *Dimorphotheca spectabilis* and *Zeyheri*.

Rabbit No. 6, weight 1,700 gm. received 30 mgm. of glucoside dissolved in water, followed by 0.25 gm. of *D. spectabilis* powder suspended in about 10 c.c. of water. No symptoms within 15 minutes. A further dose of 100 mgm. of glucoside and 0.25 gm. of plant powder was then administered. No symptoms within 1 hour.

Conclusion: As in the case of amygdalin, the *Dimorphotheca* glucoside when administered in the unhydrolysed condition, is not toxic to rabbits in doses greater than 1 M.L.D. in terms of hydrogen cyanide.

IV. Tests upon the *Dimorphotheca* glucoside when hydrolysed prior to feeding.

90 mgm. of glucoside was dissolved in 10 c.c. of buffer solution (pH 6.0), about 1 mgm. of *D. spectabilis* powder added and the mixture left in a stoppered flask overnight.

Rabbit No. 7, weight 1,650 gm., received this mixture by stomach-tube at 12 noon on 2.3.32. 12.02 p.m. very laboured respiration, weak; 12.04 p.m. head falls to one side, animal weak and comatose; 12.07 p.m. died.

Post-mortem appearance: General cyanosis; hyperaemia of lungs, liver, kidneys and spleen; both ventricles of heart greatly dilated; slight intestinal catarrh.

Conclusion: Typical hydrogen cyanide poisoning. The glucoside is markedly toxic when enzymic hydrolysis, with liberation of free hydrogen cyanide, is allowed to take place prior to administration. The symptoms and post-mortem appearances are in every way identical with those seen in typical cases of poisoning from the ingestion of *Dimorphotheca spectabilis* or *Zeyheri*.

REFERENCES.

- ALSBERG, C., and BLACK, O. (1916). The separation of autogenous and added hydrocyanic acid from certain plant tissues and its disappearance on maceration. *Jnl. Biol. Chem.*, Vol. 25, pp. 133-140.
- BRÜNNICH, J. (1903). Hydrocyanic acid in Fodder plants. *Jnl. Chem. Soc.*, Vol. 83, p. 788.
- DUNSTON, W. R., and HENRY, T. A. (1904). Cyanogenesis in Plants. Part III. On Phaseolunatin, the cyanogenetic glucoside of *Phaseolus lunatus*. *Proc. Roy. Soc.*, Vol. 72, pp. 285-294.
- DUNSTON, W. R., HENRY, T. A., and AULD, S. J. M. (1906 a). Cyanogenesis in Plants, Part IV. The occurrence of Phaseolunatin in common flax (*Linum usitatissimum*). *Proc. Roy. Soc.*, Vol. 78, pp. 145-152.
- DUNSTON, W. R., HENRY, T. A., and AULD, S. J. M. (1906 b). Cyanogenesis in Plants, Part V. The occurrence of Phaseolunatin in Cassava (*Manihot aipi* and *Manihot utilissima*). *Proc. Roy. Soc.*, Vol. 78, pp. 152-158.
- DUNSTON, W. R., HENRY, T. A., and AULD, S. J. M. (1907). Cyanogenesis in Plants, Part VI. On Phaseolunatin and the associated enzymes in Flax, Cassava and the Lima Bean. *Proc. Roy. Soc.*, Vol. 79, pp. 315-322.
- FISCHER, E. (1894). Einfluss der Configurationen auf die Einwirkung der Enzyme II. *Ber.* Vol. 27, pp. 3479-3480.
- FISCHER, E., and ANGER, G. (1919). Synthese des Linamarins und des Glycollonitrile-cellosides. *Ber.* Vol. 52, pp. 854-868.
- JORISSEN, A., and HAIRS, E. (1891). *Bull. Acad. Roy. Belg.*, series 3, Vol. 21, p. 529.
- Official and Tentative Methods of Analysis, Ass. Official Agric. Chemist. Washington, 1930.
- PHILLIPS, E. P. (1926). A Preliminary list of the known poisonous plants found in South Africa. Pretoria, 1926.
- ROSENTHALER, L. (1922 a). *Schweiz. Apoth. Zeit.*, Vol. 60, pp. 234-
- ROSENTHALER, L. (1922 b). Über eine Glucosidase aus *Dimorphotheca Ecklonis* D.C. *Fermentforsch.*, Vol. 6, pp. 197-199.
- STEYN, D. G. (1929). Recent Investigations into the Toxicity of known and unknown poisonous plants in the Union of South Africa. 15th Rept. *Dir. of Vet. Educ. and Res.*, pp. 777-803.
- TREUB (1905). *Ann. Jard. Bot. Buitenzorg*, Series 2, Vol. 4, p. 86.
- VIEHOEYER, A., and JOHNS, C. (1915). Estimation of Small quantities of Hydrocyanic acid. *Jnl. Amer. Chem. Soc.*, Vol. 37, pp. 601-607.

Section VIII.

Animal Industry.

J. E. DUERDEN, Growth of Wool in the Merino.
C. A. MURRAY,
AND P. S. BOTHA

J. E. DUERDEN Staple Length, Variation and Distribution in
AND E. W. the Fleece of the Merino.
PALMER.

Growth of Wool in the Merino.

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CONTENTS.

- I. Introduction.
 - II. Methods.
 - III. Rate of Growth and Weights: Group A, Group B, Group C.
 - IV. Correlations: Body Weight and Fleece Weight; Fleece Weight and Fibre Length; Body Weight and Wool Length; Fibre Length and Thickness.
 - V. Summary.
 - VI. References.
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I. INTRODUCTION.

THE rate of growth of wool varies with the individual sheep, and according to season, climate, pasturage, and other conditions. In farming practice it is generally held to grow more rapidly after shearing than before; the winter growth is said to be slower than that during the summer; and a shearing of twelve months is expected to weigh less than two shearings of six months. Early investigators, such as Rohde (1857), Stohmann (1873), Heyne (1916), and Gartner (1924), hold that wool grows almost twice as rapidly during the first six months after shearing as in the next six months. Later investigators, however, like Nordmeyer (1927), Hardy and Tennyson (1930), Hackedorn and Sotola (1929), and more recently, Burns (1931), report a comparatively uniform growth throughout the year. Some time ago the Potchefstroom School of Agriculture arranged an investigation in which the wool was to be actually measured month by month during its growth, and its rate of increase considered in connection with the various internal and external conditions.

The investigation was begun in May, 1928, and continued until March, 1930. About 30 Merino wethers were maintained under farm conditions, and clippings taken at regular intervals of 28 days from the front and hind legs of each (Fig. 1). The first experiment lasted for twelve months and another was continued for a second twelve months, with a different group of wethers. Later, a third lot was selected from which samples were taken for only six months. The sheep were weighed month by month (28 days), and records made of the climatic and grazing conditions at Potchefstroom. The tabulations and most of the wool measurements were made at the Wool Research Laboratory, Grahamstown.

II. METHODS.

The sheep were pastured on grass veld, supplemented by artificial feeding during periods of scarcity. The groups consisted of individual wethers, as much alike as was possible in a selection made from a mixed flock of different ages. The actual weighings necessarily included both the body weight and the fleece weight. The average body weights, as given in the tables, were obtained by deducting the estimated fleece weight for each period from the total sheep weight. The total fleece weight is that given at the final shearing, and the approximate monthly weights were obtained as follows: if W represents the fleece weight of the eleven months, then $\frac{W}{11}$ may be taken as

the average for 1 month, $\frac{W}{11} \times 2$ the weight for 2 months, and so on.



Fig. 1.—Wether showing position of the patches on the front and hind legs from which the wool samples were taken at 28-day intervals.

The samples were taken from a patch about two inches square on both the fore and hind limb of one side, as shown in Fig. 1, the wool having been completely removed at the beginning by means of a safety razor.* In Group A a small portion of skin with the fibres attached was cut monthly from each patch. Considering the smallness of the piece of skin excised, and the well-known rapid recovery of the sheep's skin from cuts, as in shearing, the effects on the surrounding skin from hyperaemia need scarcely be considered. In Groups B and C, adjacent separate staples were clipped as close to the skin as possible by means of fine scissors.

* It is popularly believed that shaving, as in human beings, will stimulate fibre growth. Danforth (1925), however, holds that this is not the case, and gives strong evidence in support of his contention.

TABLE 1.—GROUP A.
AVERAGE STRAIGHT FIBRE LENGTH MONTH BY MONTH AND MONTHLY RATE OF GROWTH (MM.).

Area.	Winter.				Summer.							Average Increase.	
	15.6.28.	13.7.28.	10.8.28.	7.9.28.	5.10.28.	2.11.28.	30.11.28.	28.12.28.	25.1.29.	22.2.29.	22.3.29.	28 Days.	Per Day.
Front leg.....	10.9	23.2	30.0	37.5	45.3	57.9	69.6	79.6	97.9	107.4	117.5	10.7	0.38
Hind leg.....	10.4	23.1	29.0	35.5	42.4	55.5	67.4	77.2	90.8	100.5	111.3	10.1	0.36
Increase :													
Front leg.....	10.9	12.3	6.8	7.5	7.8	12.6	11.7	10.0	18.3	9.5	10.1	10.7	0.38
Hind leg.....	10.4	12.7	5.9	6.5	6.9	13.1	11.9	9.8	13.6	9.7	10.8	10.1	0.36
Average increase.....	10.7	12.5	6.4	7.0	7.3	12.5	11.8	9.9	15.9	9.6	10.5	10.4 ± .55	0.37
Average winter growth (straight length) for 28 days, 8.76 ± .72 mm.													
Co-efficient of variability, monthly increases : 26.0 per cent.													
Average summer growth for 28 days, 11.77 ± .60 mm.													
Difference between average summer and winter growths = 3.01 ± .43 mm.													
AVERAGE MONTHLY WEIGHTS, 28 SHEEP (lb.).													

Area.	Winter.				Summer.						Average.	
	15.6.28.	13.7.28.	10.8.28.	7.9.28.	5.10.28.	2.11.28.	30.11.28.	28.12.28.	25.1.29.	22.2.29.		22.3.29.
Weights.....	77.0	79.1	79.3	79.0	73.4	80.6	82.4	86.1	89.9	90.6	91.0	82.6
Fleece weight (estimated);	0.8	1.5	2.3	3.1	3.8	4.5	5.3	6.1	6.8	7.5	8.3	4.5
Carcass weights.....	76.2	77.6	77.0	75.9	69.6	76.1	77.1	80.0	83.1	83.1	82.7	78.0 ± .78
Average winter body weight, 75.3 ± .87 lb.												
Co-efficient of variability, body weights : 4.9 per cent.												
Co-efficient of correlation : Body weight : rate of growth of wool = .544 ± .14.												
Average summer body weight, 80.3 ± .79 lb.												
Difference between average summer and winter body weights = 5.0 ± .69 lb.												

Since the investigation was commenced the difficulty of procuring representative staple samples, even of a small area, has been emphasized by Roberts (1930) and Burns (1931), and is always manifest. The samples taken as described may not be altogether satisfactory for the study of individual sheep, on account of the possible differences in adjacent staples. When it is realized, however, that the averages are based upon approximately 56 samples for each group, they may be accepted as justifying the general conclusions reached.

In making the length measurements for Group A, 20 fibres were selected at random from each sample. The straight length of each was ascertained by holding the unstretched fibre, with the crimps just removed, along a millimetre scale by means of fine smooth-tipped forceps. This method is followed by Burns (1931), and is found to be satisfactory for comparative estimations. Roberts (1930) also uses the method for obtaining the straight length of the fibres in his estimations of fibre thickness by the weight-length method. The staple length of the clippings in Group B was measured directly by means of the scale. The measurements of Group C were carried out at Potchefstroom. In these the staple length was first ascertained on the live sheep by means of a graduated steel rod, in form much like an ordinary lead pencil, and the same staple was afterwards shorn and measured directly with the scale. As shown in Table 3, the rod measurements in all cases are higher than those from the clippings, a result of the heavy rod indenting the soft skin. On account of this objection the rod has since been discarded, though, in later investigations, a very light tapering steel scale has been found to be satisfactory.

III. RATE OF GROWTH AND WEIGHTS.

At the beginning Group A consisted of 31 wethers which were shorn on March 27th, 1928. The patches on the legs were selected and shaved on the 18th May, when the sheep had become adapted to their surroundings. After 28 days a small piece of skin with the wool attached was cut from each of the patches. The same operation was carried out for the eleven succeeding 28-day periods until the 22nd March, 1929, when the last clipping was taken, the sheep being shorn on the 27th. The weighings were made at the same time as the wool samples were procured. During the year three sheep died, so that the full data obtained only apply to 28 individuals.

Table 1 gives the average straight length of the fibres from the two patches month by month, and the average monthly increase; also the average sheep weights and the estimated fleece and body weights for the corresponding periods. The average weights for each sheep and its final fleece weight are given in Table 4.

The table shows that the monthly increase is somewhat irregular for both the fore- and hind-leg, the average of the two ranging from 6.4 mm. during the July-August period to 15.9 mm. during the December-January period. The co-efficient of variability is thus very high, 26.0 per cent. Burns (1931) likewise found much variation in estimating the rate of growth by the above method. The growth on

TABLE 2.—GROUP B.
AVERAGE STAPLE LENGTH MONTH BY MONTH AND MONTHLY RATE OF GROWTH (mm.).

Area.	Winter.					Summer.					Average Increase.		
	Winter.					Summer.							
	4.6.29.	2.7.29.	30.7.29.	27.8.29.	24.9.29.	22.10.29.	19.11.29.	17.12.29.	14.1.30.	11.2.30.		11.3.30.	28 Days.
Front leg.....	5.7	11.1	15.3	20.8	26.3	33.3	39.0	44.9	50.4	54.5	61.3	5.6	0.20
Hind leg.....	5.1	10.6	13.6	18.8	24.2	30.6	35.7	42.0	45.9	50.6	57.5	5.2	0.18
Increase :													
Front leg.....	5.7	5.4	4.2	5.5	5.5	7.0	5.7	5.9	5.5	4.1	6.8	5.6	0.20
Hind leg.....	5.1	5.5	3.0	5.2	5.4	6.4	5.1	6.3	3.9	4.7	6.9	5.2	0.18
Average increase.....	5.4	5.4	3.6	5.3	5.4	6.7	5.4	6.1	4.7	4.4	6.8	5.4 ± .18	0.19

Average winter growth (staple length) for 28 days : 5.02 ± .07 mm.
Co-efficient of variability, monthly increases : 16.6 per cent.

Average summer growth for 28 days, 5.70 ± .25 mm.
Difference between average summer and winter growths : .68 ± .02 mm.

AVERAGE MONTHLY WEIGHTS, 27 SHEEP (LB.).

AVERAGE MONTHLY WEIGHTS, 27 SHEEP (LB.).

Area.	Winter.					Summer.							Average.
	4.6.29.	2.7.29.	30.7.29.	27.8.29.	24.9.29.	22.10.29.	19.11.29.	17.12.29.	14.1.30.	11.2.30.	11.3.30.		
Weightings.....	87.5	84.1	79.1	86.9	85.8	93.8	101.7	97.2	99.2	97.6	100.0	92.1	
Fleece weight (estimated)	0.8	1.5	2.3	3.0	3.8	4.5	5.3	6.1	6.8	7.5	8.3	4.5	
Carcass weights.....	86.7	82.6	76.8	83.9	82.0	89.3	96.4	91.1	92.4	90.1	91.7	87.6±1.10	
Average winter body weight: 82.4 ± .83 lb.													
Average summer body weight: 91.8 ± .63 lb.													
Co-efficient of variability, body weights: 6.2 per cent.													
Difference between average summer and winter body = weights 9.4 ± .52 lb.													
Co-efficient of correlation: body weight: rate of growth of wool = .455 ± .10.													

TABLE 3.—GROUP C.

AVERAGE STAPLE LENGTH MONTH BY MONTH AND MONTHLY RATE OF GROWTH AS MEASURED BY STEEL ROD ON SHEEP (R)
AND AFTER CLIPPING (C).

Area.	Summer.												Average Increase.			
	22.10.29.		19.11.29.		17.12.29.		14.1.30.		11.2.30.		11.3.30.		28 Days.		Per Day.	
	R.	C.	R.	C.	R.	C.	R.	C.	R.	C.	R.	C.	R.	C.	R.	C.
Front leg.....	6.0	6.0	12.8	12.2	21.2	19.1	25.2	24.5	30.6	29.2	40.5	36.1	6.9	6.0	0.25	0.22
Hind leg.....	4.9	5.6	10.9	11.4	18.6	16.9	23.8	22.6	30.6	27.6	26.6	32.3	6.4	5.3	0.23	0.20
Increase:																
Front leg.....	—	—	6.8	6.2	8.4	6.9	4.0	5.4	5.4	4.7	9.9	6.9	6.9	6.0	0.25	0.22
Hind leg.....	—	—	6.1	5.7	7.7	5.6	5.2	5.7	6.7	4.9	6.1	4.8	6.4	5.3	0.23	0.19
Average increase.....	—	—	6.4	5.9	8.0	6.2	4.6	5.5	6.0	4.8	8.0	5.8	6.6	5.6	0.24	0.21
Average summer growth (staple length) for 28 days, by Rod, $6.65 \pm .39$ mm.; by Clippings, $5.65 \pm .14$ mm. Co-efficient of variability, monthly increases: Rod, 19.4 per cent.; Clippings, 8.5 per cent.																
AVERAGE MONTHLY WEIGHTS, 27 SHEEP (LB.).																
Area.	Summer.												Average.			
	22.10.29.		19.11.29.		17.12.29.		14.1.30.		11.2.30.		11.3.30.					
Weights.....	90.2		99.3		94.6		98.1		94.3		96.6		95.5			
Fleece weight (estimated).....	0.9		1.7		2.6		3.5		4.3		5.2		3.0			
Carcass weights.....	89.3		97.6		92.0		94.6		90.0		91.4		$92.5 \pm .75$			

Average summer body weight: $92.5 \pm .75$ lb.
Co-efficient of correlation: body weight; rate of growth of wool = $.37 \pm .26$.
Co-efficient of variability, body weights: 3.0 per cent.

the fore-leg is slightly greater than that on the hind-leg, as is usually found to be the case; the difference between the two on the final clipping, 22nd March, is 6.2 mm., a percentage of 5.6.

The growth for the first five periods took place during the winter season, May to October, and gives an average of 8.76 mm. for each 28 days, whereas that during the summer months, October to March, gives the higher average of 11.77 mm. for the same number of days, a difference of 3 mm. The 28-day average for the whole period, 10.4 mm. compares very closely with 10.66 mm. per calendar month found in a previous experiment which was conducted at the Grootfontein School of Agriculture under approximately similar conditions (1931). The body weights show a corresponding difference, the average winter weight being 75.3 lb. and the average summer weight 80.3 lb. The variability of the body weights is not, however, so great as that of the wool lengths, being represented by 4.9 per cent.

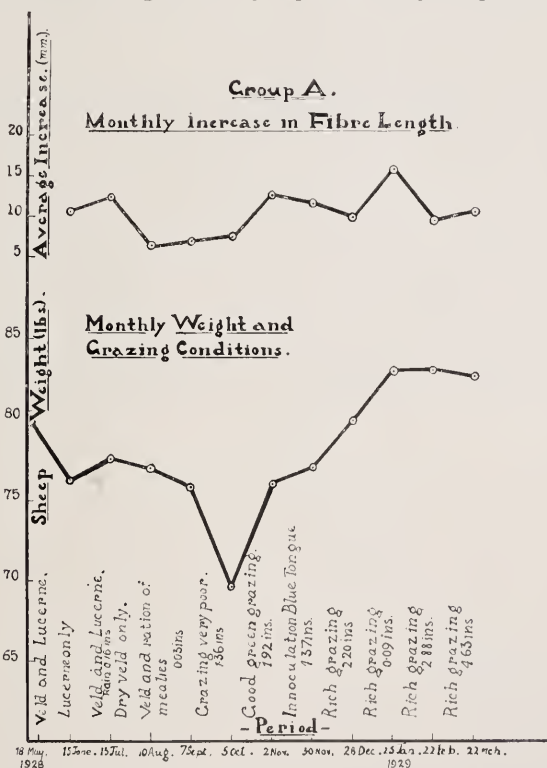


Fig. 2.—Diagram giving (1) the monthly increases in straight fibre length, and (2) the monthly weights of the sheep in Group A, during a period of eleven 28-day intervals. The grazing conditions and monthly rainfall are added. The weights of the sheep in this and in Figs. 3 and 4 represent only the body weight, the estimated weight of the fleece, increasing month by month, being deducted from the actual weighings.

The results presented in the table are graphically shown in Fig 2. The upper graph represents the monthly increase in fibre length, the lower the monthly weights, the grazing conditions being added for each period. In a general way the body weights decrease during the winter months as far as October, and afterwards gradually rise to the middle of summer, remaining the same to the end of March, the summer grazing being more favourable than the winter.

The rate of increase in the fibre length of the fleece is somewhat less during the winter period than during the summer, but no close comparison can be drawn with the variations in body weight and in the grazing conditions.

Group B.

The group consisted of 32 wethers at the beginning of the experiment, of which 27 were left at the end of the twelve months. Shearing took place on the 27th March, 1929, and on the 7th May the

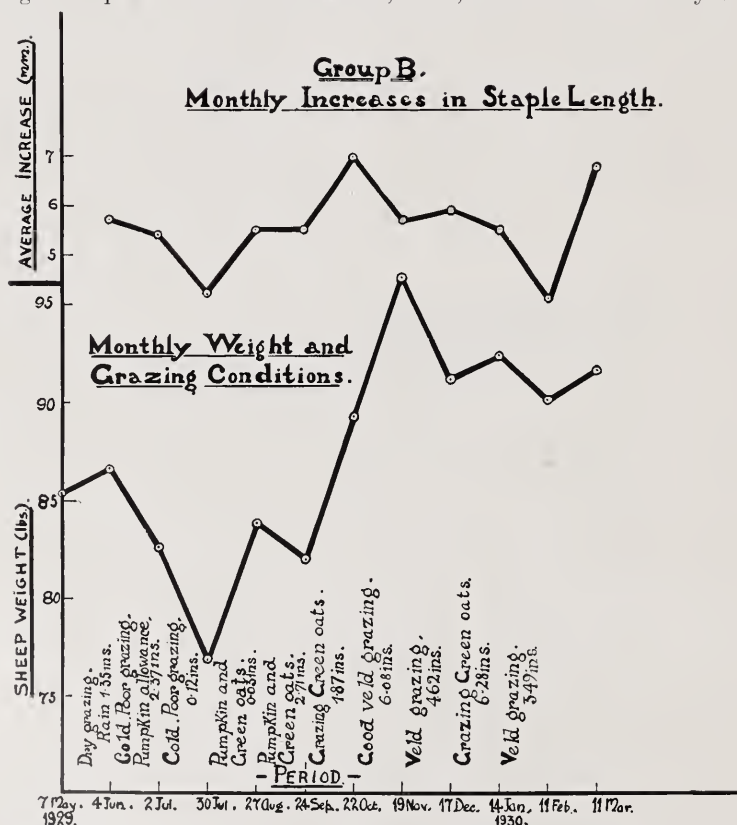


Fig. 3.—Diagram showing (1) increases in staple length month by month, and (2) the varying monthly sheep weights in Group B. The grazing conditions and rainfall are also given.

patches were selected on the front and hind leg, as in Group A. The first samples were taken on the 4th June and afterwards at regular intervals of 28 days, the last clipping being taken on the 11th March, 1930. In taking the samples a small staple of wool was clipped with fine scissors as close to the skin as possible, in place of removing a bit of the skin with the wool attached, as in the previous group.

The measurements and weighings are given in Table 2, but it must be noted that the length measurements refer to staple length, whereas those in Table 1 are straight fibre lengths. The growth again shows a slight increase on the front leg as compared with the hind, the difference, 3.8 mm. representing a percentage of 6.6. The monthly increases in staple length present a high variability (16.6 per cent.), though not so high as the straight lengths in Group A (26.0 per cent.). The average winter growth (5.02 mm.) is slightly less than the summer (5.70 mm.), representing a difference of 0.68 mm.

The difference in the length between winter and summer corresponds closely with the difference in the average body weight during the winter and summer, namely, 82.4 lb. and 91.8 lb. respectively, indicating that the sheep were in a lowered state of nutrition during the winter. In his experiments Burns (1931) found the weight to be remarkably uniform throughout the year, but to be affected by the lambing season (ewes) and the abundance or lack of green food.

The differences are graphically shown in Fig. 3, representing the monthly increases in staple length and the monthly weighings, along with the veld conditions. The lowest weighings were given for the July period, which may be taken as the middle of winter, and this nearly corresponds with the lowest rate of monthly growth of the wool. The highest summer weighings, November period, practically correspond with the highest rate of wool growth.

TABLE 4.

AVERAGE BODY WEIGHT DURING TWELVE MONTHS AND TOTAL FLEECE WEIGHT AND STRAIGHT FIBRE LENGTH.

GROUP A.—28 SHEEP.

Sheep No.	Average Weight Body and Fleece.	Average Body Weight.	Fleece Weight.	Straight Fibre Length.
	lb.	lb.	lb.	mm.
21	98.1	93.4	8.5	108.7
36	98.3	93.3	8.0	110.3
66	97.7	92.5	9.5	113.3
50	96.2	90.3	11.0	125.5
19	94.0	89.3	8.75	117.4
43	92.9	87.9	7.75	107.1
62	92.4	87.2	9.5	120.3
57	88.6	85.9	6.75	128.3
37	88.7	83.8	9.0	123.9

GROWTH OF WOOL IN THE MERINO.

GROUP A.—28 SHEEP—*continued*.

Sheep No.	Average Weight Body and Fleece.	Average Body Weight.	Fleece Weight.	Straight Fibre Length.
22	83·7	79·4	7·75	124·9
39	83·1	78·4	8·5	90·8
17	82·2	77·8	8·0	100·0
1	80·3	77·5	7·75	111·5
38	82·0	76·9	9·25	136·3
52	80·7	76·4	8·0	115·1
20	79·7	76·4	6·0	122·0
59	80·0	75·9	7·5	97·4
41	79·7	75·2	8·0	126·4
42	79·2	74·3	8·75	117·0
274	76·2	72·5	8·5	134·0
30	75·7	70·4	9·5	102·0
56	73·2	69·1	7·5	106·3
64	72·8	68·7	7·5	120·8
15	72·2	67·8	8·0	102·8
31	72·1	67·7	8·0	109·4
51	71·1	66·7	8·0	102·4
273	69·3	64·9	8·0	134·1
65	66·4	61·5	9·0	114·8
Mean.....	82·3	77·9	8·3	115·2
E. of Mean....	$\pm 1·14$	$\pm 1·20$	$\pm 0·13$	$\pm 1·52$
S. Dev.....	$\pm 8·95$	$\pm 9·08$	$\pm 0·98$	$\pm 11·55$
Co-eff. Var.....	10·87%	11·66%	11·81%	10·03%

Co-eff. Cor: (a) Body weight: Fleece weight = $\cdot 256 \pm \cdot 119$.(b) Body weight: Fibre length = $\cdot 053 \pm \cdot 127$.(c) Fleece weight: Fibre length = $\cdot 093 \pm \cdot 126$.*Group C.*

Group C started with 32 wethers, shorn on the 27th September, 1929. The patches were shorn on the 1st October, 1929, and the first set of clippings and weights taken on the 22nd October, and the last on the 11th March, 1930, six months later, when 29 sheep were left. The measurements were all made at Potchefstroom, both by the rod method on the live sheep and from clippings.

The summary for the six months is shown in Table 3, and gives the average staple length at each clipping and the monthly rate of growth, as taken both by the steel rod and from clippings. The growth as measured by the rod is shown to be longer than that of the clipped staple, the result of the heavy rod indenting the skin. The co-efficients show both measurements to be very variable, those from the rod being more so than those from the clippings.

Both methods again indicate a longer growth on the front leg than on the hind; the average growth on both patches for every 28 days is 6·65 mm. as given by the rod, and 5·65 mm. as measured after clipping.

The wool was grown during the same summer as the summer growth of Group B, the average growth of the two being almost exactly the same, namely, 5.70 mm. and 5.65 mm. Also the variations in the sheep weights resulting from the grazing conditions, and the differences in the monthly rate of wool growth, closely correspond with those of Group B. The graphs in Figures 3 and 4 are much alike, showing the inter-dependence of the rate of wool growth on the nutrition conditions of the sheep.

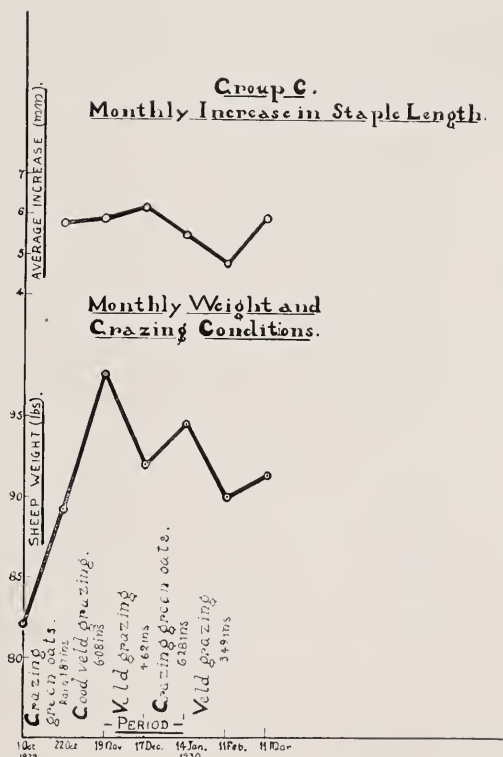


Fig. 4.—Variations in the monthly rate of growth in staple length, and the monthly sheep weights for Group C, for a period of only six 28-day intervals. Grazing conditions and rainfall added.

IV. CORRELATIONS.

The data in the previous section refer to body and fleece weights and to fibre length measurements, and were collected primarily to afford evidence bearing on the rate of growth of wool and its response to seasonal, climatic and pasture conditions. The same details can, however, be employed to examine certain other relationships.

GROWTH OF WOOL IN THE MERINO.

TABLE 5.

AVERAGE BODY WEIGHT DURING TWELVE MONTHS AND TOTAL FLEECE WEIGHT AND STAPLE LENGTH.

GROUP B.—27 SHEEP.

Sheep No.	Average Weight Body and Fleece.	Average Body Weight.	Fleece Weight.	Staple Length.
	lb.	lb.	lb.	mm.
58	107.9	103.7	7.87	55.9
24	108.2	102.8	10.00	84.9
576	101.2	97.6	6.69	60.8
18	100.7	96.5	7.67	54.8
596	98.2	93.6	8.56	59.6
4	97.2	93.4	6.81	54.7
599	96.9	91.9	9.62	57.9
575	96.5	91.6	8.92	61.7
3	97.6	91.0	10.56	59.6
44	94.7	89.9	8.81	59.1
32	95.9	89.6	10.19	52.7
585	92.5	88.9	7.00	50.1
592	92.6	88.4	7.69	62.2
5	93.4	88.1	9.81	56.5
54	91.7	86.7	9.00	55.4
460	90.8	86.4	9.37	65.9
47	91.2	86.0	9.69	57.7
574	89.4	85.1	7.94	61.2
29	89.7	84.5	9.62	59.7
579	88.3	84.4	7.31	63.2
46	88.5	84.0	8.37	62.0
34	85.9	82.0	7.19	57.8
63	83.5	79.6	7.12	56.0
8	78.0	74.3	6.87	61.9
67	79.1	74.2	8.87	59.8
584	74.3	70.6	6.81	50.5
7R310	71.1	67.5	6.56	62.8
Mean.....	92.4	86.8	8.33	59.0
E. of Mean....	± 1.15	± 1.12	± 0.16	± 0.08
S. Dev.....	± 8.85	± 8.61	± 1.22	± 6.27
Co-eff. Var.....	9.58%	9.91%	14.65%	10.63%

Co-eff. Cor.: (a) Body weight : Fleece weight = $.346 \pm .114$.(b) Body weight : Staple length = $.238 \pm .122$.(c) Fleece weight : Staple length = $.458 \pm .102$.

In Tables 4, 5 and 6 are given the averages of the monthly weighings of each sheep (body and fleece) in the three Groups A, B and C. The fourth column in each gives the fleece weight of each sheep on shearing at the conclusion of the experiment. The body weights in the third column represent the average weight during the twelve months after the estimated weight of the fleece at each period has been deducted. Straight fibre or staple lengths are given in the

last columns. In the present section attention is directed to the correlations of body weight and fleece weight, body weight and fibre length, and fleece weight and fibre length, as also those of fibre length and thickness.

In breeding practice farmers expect certain attributes of the sheep and fleece to be correlated with one another, that is, to be related in a constant manner such that if one varies so does the other; in striving to increase or decrease the one attribute they tend to increase or diminish the other. Thus, it is generally accepted that in increasing the length of the wool the thickness will also be increased, while the density will become less; increasing the fineness and quality decreases the length, and the weight of the fleece also. Mr. G. J. Schuurman (1929), Principal Sheep and Wool Officer, has given some attention to these correlations as between the wrinkly and plain-bodied types of sheep. His conclusions may be expressed as follows:—

Wrinkly Type.

Small body.
Weak constitution.
Short on legs.
Slow maturing.
Closed face.
Wrinkles on body.
Over abundance of yolk.
Short, dense, fine wool.

Plain-bodied Type.

Big body.
Strong constitution.
Long on legs.
Early maturity.
Open face.
No folds on body.
Insufficiency of yolk.
Long medium to strong wool
lacking in density.

At the present time no sharp distinction separates these two types of sheep in South Africa, for much crossing has taken place, and the characteristics mentioned may occur in all degrees of combination. The relationships as presented are, however, based upon a wide experience, and are largely accepted in practice, though they have never been definitely established by actual measurements. While they may hold in a general way, modern genetics would lead one to expect that practically any combination of characters can be brought together in a single animal, that is, one attribute is not necessarily exclusive of another.

Body Weight and Fleece Weight.—In general a big carcass of the plain-bodied type of Merino is expected to give a long, medium or strong wool as contrasted with a short, dense, fine wool from a small carcass of the wrinkly type, the total fleece weight in the former being greater than in the latter. In an ordinary mixed flock of sheep, however, no close relationship appears to hold between the size or weight of a sheep and the weight of its fleece. It is clearly of some practical significance to know definitely whether any connection does exist, for a bigger animal may be expected to consume more food than a smaller one. All other attributes being the same, a heavier fleece brings in more returns than a lighter one, and the breeder is concerned in knowing whether the fleece weight is influenced by the body weight.

TABLE 6.

AVERAGE BODY WEIGHT DURING SIX MONTHS AND TOTAL FLEECE WEIGHT AND STAPLE LENGTH.

GROUP C.—29 SHEEP.

Sheep No.	Average Weight Body and Fleece.	Average Body Weight.	Fleece Weight.	Staple Length.
	lb.	lb.	lb.	mm.
570	108·1	105·9	5·6	36·1
50	109·4	105·5	6·9	39·5
66	107·3	104·3	5·1	30·6
19	106·9	104·1	4·9	30·7
591	106·4	102·7	6·5	26·2
588	103·3	100·5	5·0	40·0
595	101·7	98·4	5·8	27·0
43	100·9	97·9	5·1	38·6
593	99·6	96·8	4·9	28·5
20	96·7	94·3	4·1	34·0
581	97·9	94·3	6·2	27·0
580	96·8	93·8	5·2	34·4
22	94·7	91·5	5·6	40·0
567	93·4	90·7	4·7	47·1
1	93·2	90·4	4·9	31·2
37	93·3	90·1	5·7	34·2
41	93·2	90·0	5·6	34·6
59	92·7	89·5	5·6	24·8
23	82·4	89·2	5·5	35·6
26	91·2	88·0	5·7	34·3
39	86·9	84·1	4·8	31·0
31	86·1	83·1	5·1	35·0
56	85·5	82·5	5·2	31·7
30	85·4	82·4	5·2	32·0
52	85·0	82·4	4·6	32·1
274	80·7	78·1	4·5	38·7
560	79·7	76·7	5·1	44·0
563	78·7	75·9	4·8	33·0
65	77·8	75·7	3·7	30·0
Mean.....	93·6	91·0	5·2	33·2
E. of Mean....	±1·17	±1·13	±0·79	±0·60
S. Dev.....	±9·36	±9·05	±0·63	±4·78
Co-eff. Var....	10·0%	9·94%	12·04%	14·40%

Co-eff. Cor.: (a) Body weight : Fleece weight = $\cdot 524 \pm \cdot 091$.(b) Body weight : Staple length = $-\cdot 025 \pm \cdot 125$.(c) Fleece weight : Staple length = $-\cdot 029 \pm \cdot 126$.

From the Tables 4, 5 and 6, Group A is seen to have a co-efficient of correlation of $\cdot 256$ between the body weight and the fleece weight, while Group B has a correlation of $\cdot 346$ and Group C of $\cdot 524$. These numbers are so small as to indicate that no definite correlation exists between the body weights and the fleece weights, though Group C may be held to suggest a light degree of correlation. Thus among small groups of sheep of the character of those here studied it can be

said that on the average a heavy sheep is just as likely to give a light fleece as a heavy one, or a light sheep is as likely to give a heavy fleece as a light fleece. Even when Group A was divided into two, and the heavier sheep placed in one group and the lighter in another, the correlations were .052 for the heavier group and -.005 for the lighter, amounts which definitely prove there is no relationship between body weight and fleece weight.

The sheep in each group may be regarded as a mixed lot, such as constitute the bulk of the flocks in South Africa, even though an effort had been made to select individuals of a similar type. Were a flock of sheep uniformly pure-bred, that is, all of the same type of body and fleece, a closer correlation would be expected. It is manifest that the degree of correlation of body and fleece may be taken as an index of the degree of uniformity or purity of a flock, a matter which is likely to become of some importance as sheep breeding is conducted on more precise genetical lines.

In 1928 Spencer, Hardy and Brandon published the results of similar observations based on 990 Rambouillet fleeces. They found the co-efficient of correlation of body weight to unscored fleece weight to be as low as $.1618 \pm .0208$, and when the fleeces were scored a co-efficient of $.0552 \pm .0213$ was obtained. They concluded that up to a body weight of about 100 lb. in Rambouillet sheep the scored fleece tends to increase in weight, and decreases slightly when the body weight of the sheep exceeds 100 lb.

Fleece Weight and Fibre Length.—Does any relationship hold between the weight of a fleece and the average length of its fibres? It would be expected that the heavier the fleece the longer would be the fibres, and the lighter the fleece the shorter the fibres. Taking the weight of the fleeces in Groups A, B and C, as given in Tables 4 to 6, and the length measurement of the fibres from the same areas, the co-efficient of correlation is shown to be .093, .458 and .029 respectively, values so low as to indicate no decided correlation between the weight of fleeces and the length of the fibres. Many factors, however, are concerned in determining the weight of a fleece, and fibre length is only one of them. Density is of high importance, and a general opinion prevails that a fine, shorter fleece is denser than a longer one, a relationship which is receiving attention; the amount of yolk and foreign matter are also factors influencing fleece weight. All these call for separate analysis before a definite answer can be given.

Body Weight and Wool Length.—The body weight of a sheep being known, it is possible to compare with it the length of the wool at shearing, and so determine if there is any constant relationship between the weight of a sheep and the length of the wool it produces. The correlation within the three groups is given in Tables 4, 5 and 6. In Group A the result is based upon the straight fibre length and in Groups B and C upon the staple length, the latter for six months' growth only. The co-efficient is .053, .238 and -.025 respectively. These low numbers prove that no fixed relationship exists between the weight of a sheep and the length of the wool grown by it, a result in agreement with what is generally held in farming practice.

As already remarked the groups are constituted of mixed flock sheep, though an effort was made to select for uniformity, and all were kept under similar pasturage conditions. Were the groups constituted of better bred, more uniform sheep a closer relationship between the weight of the sheep and the length of the wool might be expected. Data such as the above have a bearing upon matters of importance to the sheep breeder, and need to be supported by further investigations.

Fibre Length and Thickness.—A general relationship is understood to hold between the fibre length and thickness of a fleece, a long wool being usually stronger than a short one, and a short wool finer than a long one. The relationship between the individual fibres making up a single staple has been considered (Duerden and Bosman, 1931). One thousand fibres were selected at random from a staple and arranged in groups differing from one another by the one-eighth of an inch, amounting to 21 groups in all, the middle ones necessarily containing more fibres than those towards the extremes. The thickness of the fibres in each separate group was then measured, and from these the correlation between the two series of measurements, length and thickness, was calculated. This gave the high value of 0.94, which may be regarded as indicating a practically perfect correlation between length and thickness, that is, the thickness varies directly as the length; in any particular staple the longer fibres are the thicker and the shorter are the finer, or, the longer the fibre the thicker it is, the shorter the fibre the thinner.

The same result holds when staples are measured from any part of the fleece, shoulder, back, britch or belly, and may be presumed to hold for all sheep, so long as the fibres in the staple are all of one type, that is, not a mixture of wool and other fibres. But the ratio or proportion of the length to the thickness differs in wool from different parts of the same fleece, or from different sheep. Thus the fibres of a staple from the britch are shorter but also thicker than those from the shoulder; they have a smaller ratio, though among themselves the finer are the shorter.

In an ordinary case it is to be expected that breeding for fibre length will be at the expense of fineness, or, contrariwise, breeding for fineness will be at the expense of length. Is it then possible to obtain a fleece of good length and fineness combined? Only by selection. Some breeders have built up studs combining a desirable length and fineness; in other words, the ratio of fineness to length is very high. The proportion or ratio of the two attributes, length and thickness, is the real test of the desirability of a wool in this connection. It is not a fixed character for the Merino, but a peculiarity of the individual sheep and of the separate parts of the body. Most breeders would probably desire fine wool is combined with length and density. Ordinary selection for fine wool, however, means shorter wool; strains must be found in which for the same degree of fineness the average length is likewise high.

V. SUMMARY.

1. The investigation was undertaken to determine the growth of wool month by month, and as influenced by various seasonal and pastoral conditions; also the responses in the body weights. Different lots of sheep were selected as Groups A, B and C.

2. The monthly rate of wool growth gave a co-efficient of variability of 26.0 per cent. in Group A, of 16.6 per cent. in Group B, and of 9.3 in Group C. Groups A and B represent both summer and winter growths, Group C only the six summer months.

3. Some of the variation is doubtless due to the fact that adjacent staples do not always grow at the same rate, but most of it is to be associated with changes in nutrition, as reflected in the body weights and pasturage conditions.

4. The average straight-fibre growth in Group A for each 28 days is 10.4 mm., that is, slightly over a centimetre, and for each day 0.37 mm., numbers which compare closely with those obtained in previous growth experiments.

5. The average staple-length growth in Group B for each 28 days is 5.4 mm., and for each day 0.19 mm. In Group C the average summer growth is 5.6 mm. for the 28-day period, or 0.20 mm. per day, practically the same as for the summer months in Group B.

6. The average summer growth is greater than the average winter growth in Groups A and B, and the summer growth of Group C is the same as the summer growth in Group B.

7. The rate of growth on the front leg slightly exceeds that on the hind leg, confirming the general observation that a fleece gradually diminishes in length from before backwards.

8. The average body weight for Group A is 78.0 lb., the winter being 75.3 lb. and the summer 80.3 lb., a difference of 6.6 per cent.; for Group B the average is 87.6 lb., namely, 82.4 lb. for the winter weight and 91.8 lb. for the summer, a difference of 11.4 per cent.; Group C has an average summer weight of 92.5 lb., which is practically the same as the summer weight for Group B.

9. The co-efficient of variability of the individual body weights in each group is 11.66 per cent., 9.91 per cent. and 9.94 per cent. respectively; value of some significance in considering the uniformity of a flock as regards weight.

10. The co-efficient of variability of the average monthly body weighings for each of the three groups is comparatively small, namely, 4.9 per cent., 5.4 per cent. and 3.0 per cent.

11. The average unscoured fleece weight in Group A, twelve months' growth, is 8.3 lb.; for Group B, 8.33 lb.; while for Group C, six summer months' growth, it is 5.2 lb. The measure of variability of the weight of the fleeces is 11.81 per cent., 14.65 per cent. and 12.04 per cent. respectively, representing a higher individual variation than do the body weights.

12. The growth of the wool is shown to be longer in summer than in winter.

13. Both the body weights and the monthly rate of growth of the wool show a direct response to the grazing conditions of the sheep. The investigation does not, however, allow of any seasonal influence being determined apart from the pasturage conditions.

14. In the groups selected no constant relationship holds between the body weight of the individual sheep and the fleece weight; a light sheep may give a heavy fleece and a heavy sheep a light fleece. A correlation would be expected in a flock of well-bred, uniform sheep, and might be used as a measure of their uniformity.

15. Similarly, there is no constant ratio between the body weight of a sheep and the length of the wool it produces; nor apparently between the fleece weight and the wool length.

16. The results do not afford any decisive evidence of a more rapid growth during the first months after shearing in comparison with the growth during the later months. In Group C the length for the first six months after shearing is the same as that in Group B for the second six months after shearing.

REFERENCES.

- BURNS, ROBERT H. (1931). "Monthly Wool Growth of Rambouillet Ewes." *Journ. Text. Instit.*, Vol. 22.
- DANFORTH, C. H. (1925). "Hair." Amer. Med. Ass., Chicago.
- DEURDEN, J. E., and MARE, G. S. (1931). "Rate of Growth of Merino Wool Month by Month." *Farming in South Africa*, Vol. 6, No. 63.
- DUERDEN, J. E. and BOSMAN, V. (1931). "Fibre Lengths, Thicknesses and Qualities in a Single Wool Staple." *17th Rept. of the Dir. of Vety. and A.I., Union of S.A.*, pp. 771-779.
- GARTNER, R. (1924). "Schafzucht," Stuttgart.
- HACKEDORN, H., and SOTOLA, J. (1929). "Studies of Wool Growth." *Washington College Station Bulletin*, No. 237.
- HARDY, J. I., and TENNYSON, J. B. (1930). "Wool Fineness as Influenced by Rate of Growth." *Journ. Agric. Res.*, Vol. 40, No. 5.
- HEYNE, J. (1916). "Grosses Handbuch der Schafzucht auf Neuzeitlicher Grundlage." Reichenbach, Leipzig.
- NORDMEYER, H. (1927). "Untersuchungen über Entwicklung und Beschaffenheit der Wolle in der Merino-fleischschaffherde, Dubrau Diss." Berlin.
- ROBERTS, J. A. FRASER (1930). "Fleece Analysis for Biological and Agricultural Purposes." *Journ. Text. Instit.*, Vol. 21.
- ROHDE, O. (1857). "Beitrage zur Kenntniss des Wolhaares." Berlin.
- SCHUURMAN, G. J. (1929). "Handbook for Farmers in S.A." Dept. Agric., Pretoria.
- SPENCER, B. A., HARDY, J. I., and BRANDON, M. J. (1928). "Factors that Influence Wool Production with Range Rambouillet Sheep." *Tech. Bull. No. 85.*, U.S. Dept. Agric.
- STOHMANN, F. (1873). "Biologische Studien." Braunschweig.

Staple Length Variation and Distribution in the Fleece of the Merino.

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E. W. PALMER, Rhodes University College, Grahamstown.

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INTRODUCTION.

Wool classification in both buying and selling is based mainly upon length and quality, and length largely determines the use to which the wool will be applied later. In general, short wools are fine and light in weight, while long wools are strong and heavy. Length also varies with the period of growth, say, whether six or twelve months, and with the pasturage. Likewise, unless a flock is uniformly bred much variation in length occurs among the individual sheep, while age has also a marked influence.

In addition to all this the wool produced by an individual sheep differs according to its position on the body. Thus, the wool covering the neck and shoulders will generally be longer than that over the back and sides, and still longer than that over the belly, points and britch. It forms a gradient from before, backwards and downwards which may be regarded as a natural characteristic of the fleece, and is independent of the conditions to which the sheep is subjected.

STAPLE LENGTH VARIATION.

In a previous study by Duerden and Bell (1931) an attempt has been made by means of a series of charts to give the quality variation and distribution within the fleece of the merino, based on the number of crimps per inch in the staple. It is part of a more complete scheme for the estimation of fleece variability generally. In the present contribution an effort is made to determine as far as possible the natural length variation within the fleece as a whole. A dozen dried woolled skins have been procured and actual measurements carried out over one side of each, and the numbers arranged on a plan of the skin as shown in Figs. 1 to 12. As some confirmation, measurements have also been made on twenty-five lambs (hoggets) with from ten to twelve months' fleeces.

The measurements were made by means of a centimetre steel scale, tapering at one end so as to drop through the fleece to the surface of the skin. The scale is placed alongside the staple, and the length read off directly in centimetres and millimetres. As was to be expected, a certain variation occurs among adjacent staples, doubtless due to shearing irregularities, but by taking a large enough number of readings at each place a fairly reliable average is obtained. The fleece was divided into approximately equal squares by means of chalk lines, and the length taken at the intersections, all folds and pleats being avoided.

It is not claimed that the method of staple measurement gives the highest possible degree of accuracy; but when a number of estimations is taken it is shown to suffice for a general comparative study of the length of the fleece, both on the live sheep and after shearing, which is all that is called for in practice. The time involved in the more precise measurement of the individual straight fibres of the staple would render it prohibitive in practice.

It is found that a fleece can be divided into three principal areas, each having a different average staple length: *a*, the neck; *b*, the back and sides; *c*, the belly, points and britch. Measurements show the wool to be the longest over the neck, somewhat shorter over the back and sides, and shortest over the belly points and britch. The shoulder wool, usually representing the finest quality, grades into that of the neck and sides without having a distinctive length of its own. The areas are by no means sharply separated, but grade into one another and, as the figures show, the area covered by each separate average differs somewhat in different sheep. In one fleece only was the neck wool shorter than the body wool, though it may be remarked that farmers generally consider the neck wool to be shorter.

Taking the average of the twelve fleeces it is found that if the length of the neck wool be represented by 100, that of the body and sides will be represented by 89 and that of the belly, points and britch by 76, that is, the body wool is 11 per cent. shorter than the neck wool, while the belly and britch wool is 24 per cent. shorter. Expressed in another way, the neck wool is about 10 per cent. longer than that of the body and sides, and 25 per cent. longer than that of the belly and britch.

As shown, there is a constant gradation in the length from fore to hind. Furthermore a constant relationship holds between the three areas. A high degree of correlation is obtained when comparing wool from the neck regions with that of shoulder regions, its value being $.92 \pm .029$; for the neck and belly $.96 \pm .015$; and for the body and belly $.91 \pm .030$. The ratios of the length of one area to the others strongly suggest that it should be possible to predict with some assurance what will be the length of the wool from one region by knowing that from another region. Thus if the neck be 4 inches in average length, that of the body may be expected to be about 3.5 inches and that of the belly 3 inches.

The total number of measurements included in each area is as follows: neck, 589; body and sides, 1,532; belly, points and britch, 536; and these may be taken as representing approximately the respective proportions of the areas. Thus the wool covering the body and sides will be 58 per cent. of the whole, that is, roughly one-half of the total fleece, that covering the neck will be 22 per cent., that is, near one-quarter, while that over the belly, points and britch is 20 per cent., that is, almost the remaining quarter.

In skirting the shorn fleece, the wool from the belly, points and britch is usually removed and classed separately, as being inferior, and the measurements reveal that this is also the shortest wool of the fleece. Usually, however, the differences in length in the wool from the neck and body in any one fleece are not so marked as to call for separation. The manufacturer, however, desires wool to be as uniform as possible, both in length and quality, and a knowledge of its variability in length comes to have a significance, and should be taken into account in selection for stud purposes.

STAPLE LENGTH DISTRIBUTION: DESCRIPTION OF FIGURES.

In the series of twelve figures the actual staple measurements are given on the right-hand side, and in all cases it is manifest that the length becomes gradually less in passing from the neck backwards and down the sides towards the belly. No sharp line of division, however, exists between one region and another, and in places individual measurements may be longer or shorter than those immediately surrounding.

Despite the grading an attempt is made to delimit areas characterised by different length averages, with what success can be seen by following the lines of demarcation among the measurements. As far as possible each of the areas on the charts includes staples which are fairly even in length. Taking the averages of all the measurements within each particular area, as given on the left-hand side of the charts, they are found to represent real differences in the distribution of wool lengths.

The delimited areas are, however, by no means the same in extent in any two sheep, though on comparing them a general plan becomes apparent. In contrasting, say, Figs. 5 and 11, it is manifest that the longer wool of the neck may extend over a large or a small area, and similarly with the body-length wool and the belly-length

wool. In all of them, with the exception of Fig. 8, the measurements of the neck area are the highest; those over the back and sides are somewhat shorter, while those over the boundaries of the fleece, namely, the belly and points, are the shortest. The charts are therefore of value as showing the extent of the body which may be covered by different average lengths of wool.

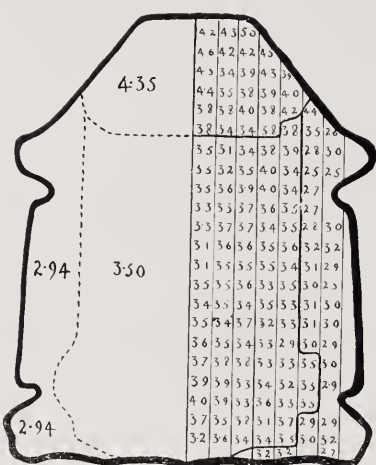


Figure 1.

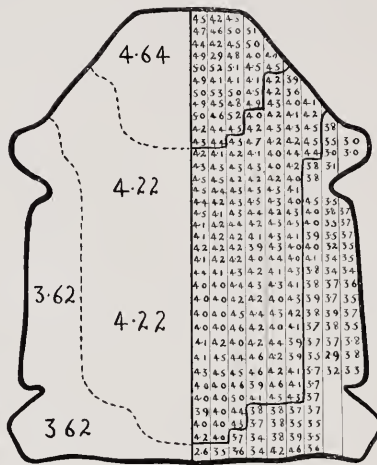


Figure 2.

Fig. 1.—As regards the staple-length distribution the fleece shown in Fig. 1 may perhaps be regarded as the simplest, and at the same time the most representative of the whole series. The neck-length wool, 4.35 cm., extends backwards and downwards to about the same distance as that included in the neck area on the live sheep. The body wool, 3.5 cm., extends from the neck region to the hind end of the body, and covers the entire back and sides; while the belly wool, 2.94 cm., extends forwards and backwards somewhat beyond the limbs.

Fig. 2.—This fleece may also be considered as representative of the series in the proportionate areas occupied by the neck, body and belly wool. The neck-length wool, 4.64 cm., occupies an area corresponding with the actual neck part of the sheep, and similarly with the body-length wool, 4.22 cm., and belly-length wool, 3.62 cm. The staples in the neck range from 4.1 cm. to 5.3 cm. with a single one of 2.9 cm., and the average, 4.64 cm., may be regarded as representative of the whole. The body-length wool ranges from 3.6 cm. to 5.0 cm., with an average of 4.22 cm., having many staples of similar length to those among the neck wool. Likewise with the belly-length wool, the general range is from 3.0 cm. to 4.0 cm., with an average of 3.62 cm. The individual staple measurements have a range from 2.6 cm. to 5.3 cm., a difference of 2.7 cm., while the averages of the areas range from 3.62 cm. to 4.64 cm., slightly over a centimetre.

Fig. 3.—The neck-length wool here extends over nearly the same area as in Fig. 2, and likewise with the body-length wool, a narrow strip of which extends in front of the shoulder compared with a broad strip in Fig. 2. The belly-wool and points-wool have practically the same distribution as before. The individual staples range from about 4.0 cm. to 6.5 cm., except extreme edges of the belly, which have staples of 3 cm., a difference of 2.5 cm.; while the averages range from 4.3 cm. to 5.58 cm., a difference of slightly over one centimetre.

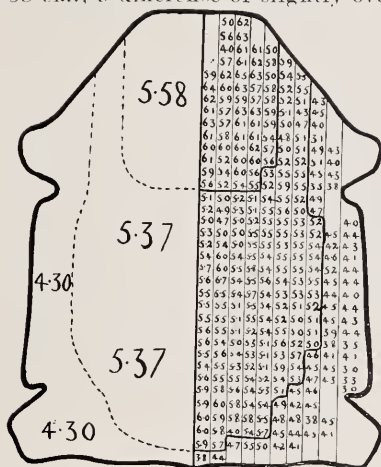


Figure 3.

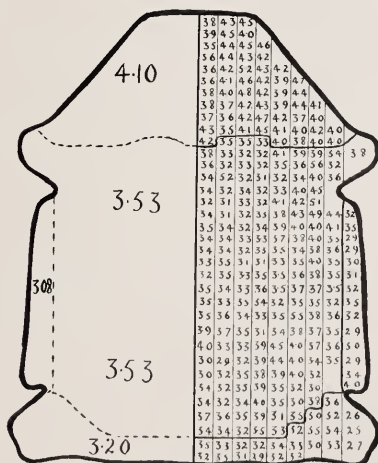


Figure 4.

Fig. 4.—The fleece shows a broad frontal expansion of the neck wool along with a broad extension of the body wool encroaching upon the belly area, the latter being proportionally narrow. The staples range approximately from 3 cm. to 4.5 cm., a difference of 1.5 cm.

Fig. 5.—The long neck-length wool extends much further backwards than usual, covering the neck and shoulder area to about the middle of the sides, that is, nearly half the whole surface of the sheep. The shorter belly-length wool is very restricted.

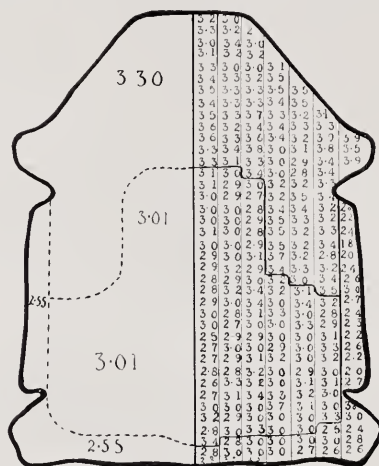


Figure 5.

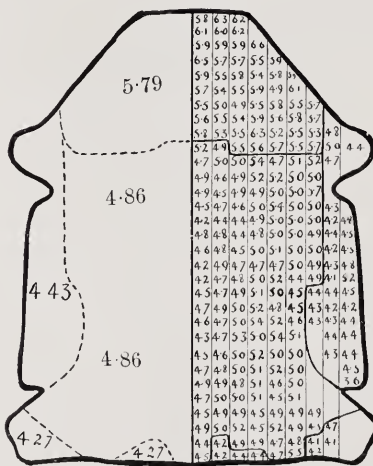


Figure 6.

Fig. 6.—The fleece shows no great variation from that represented in Fig. 1, as regards the proportional areas represented by the neck wool, but the hindermost wool tends to be the shortest of the whole fleece.

Fig. 7.—The neck-length and body-length areas are narrower than in Figs. 4 and 5, but otherwise the distribution closely resembles that in Fig. 1.

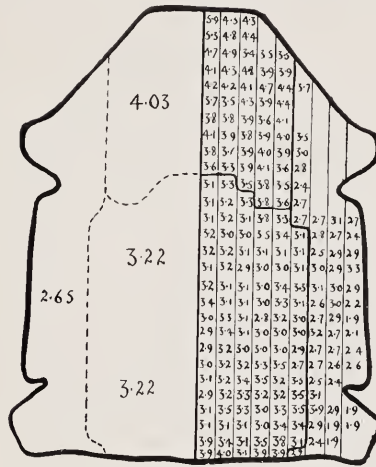


Figure 7.

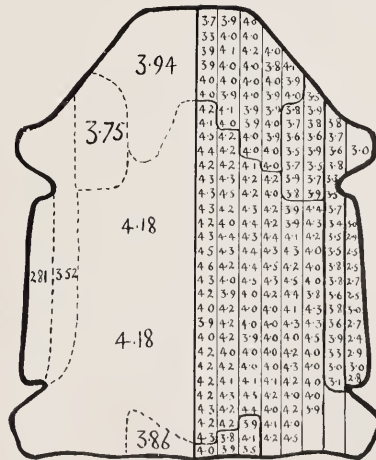


Figure 8.

Fig. 8.—The average of the front-shoulder wool is slightly less than that of the neck-length wool, but the average length of the wool over the body area is slightly greater than that of the neck area, an unusual result which may possibly be ascribed to irregularities in shearing. The belly wool is definitely divisible into two longitudinal zones. The body-length wool passes to the end of the britch, and a slightly shorter wool occurs towards the tail.

Fig. 9.—According to their length the back and sides of the fleece are divisible into two zones, the wool of the back, 3.86 cm., being shorter than that of the sides, 4.1 cm., a not unusual relationship.

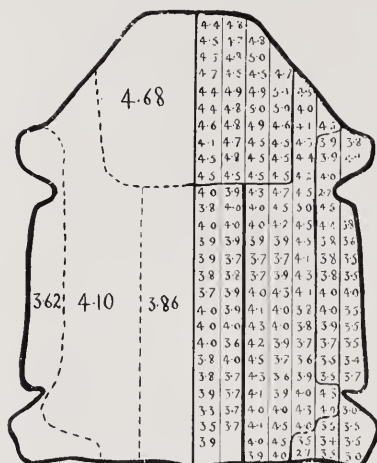


Figure 9.

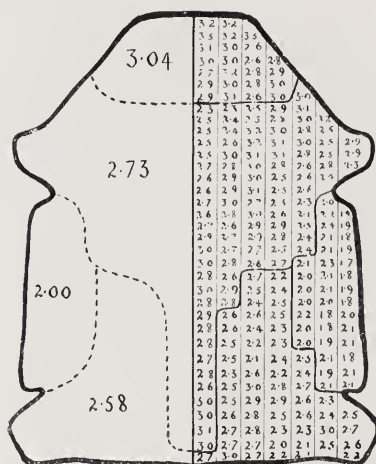


Figure 10.

Fig. 10.—The neck-length wool covers an area somewhat smaller than usual. As in Fig. 9 the back and body area can be divided into two areas of different average lengths, and the belly wool is of less extent than usual.

Fig. 11.—The fleece shows an irregular distribution in length compared with the others. The neck-length wool, 3.98 cm., is very restricted in extent, and the body-length wool is divisible into three averages, 3.48 cm., 3.30 cm. and 3.14 cm., the latter being comparable with the belly wool.

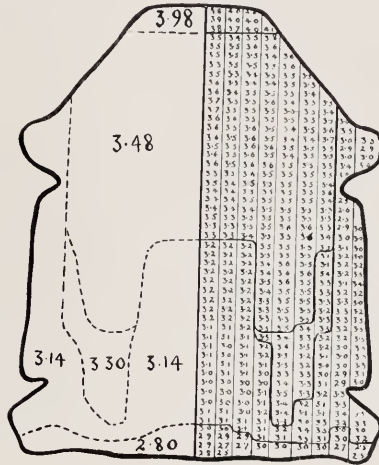


Figure 11.

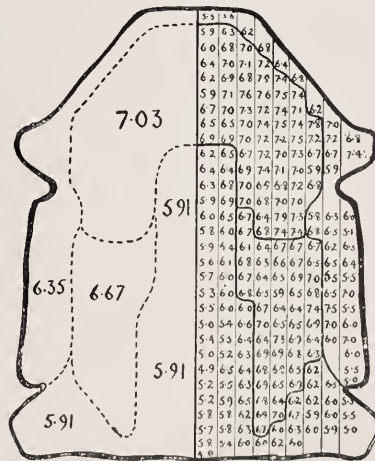


Figure 12.

Fig. 12.—The fleece displays a very unusual distribution of the staple lengths. The margin of the neck area is shorter than the dorso-lateral part, being the same as the belly wool. Neck-length wool, 7.03 cm., encroaches on the sides and occupies the front of the shoulder. A long narrow zone extends along the middle of the back distinct from the side wool and is of the same length as that of the hind region.

MEASUREMENTS ON LIVE SHEEP.

In addition to the length measurements on the dried woolled skins, a series has also been taken on live sheep, following the lines of farming practice. The method employed is the same as before, that is, the use of a light tapering centimetre scale. The sheep, 25 in number, were unshorn lambs (hoggets) with 10-12 months' wool growth, the selection being based on density and the plainness of the body, that is, its freedom from folds and pleats which are always associated with irregularities.

In the former studies the area of distribution of any particular length of wool has been considered, but in the present the areas are broadly defined and the average length of their covering ascertained. The division of the fleece into only three areas was considered, namely, neck, back and sides, and belly and points. The neck area was taken to include from the head backwards to the beginning of the shoulder, and round the sides of the neck to the mid-ventral line. The back and sides included the greater area of the fleece, the sides being delimited below by a line joining the bare axillary and inguinal patches. The belly and points comprise the whole belly, extending upwards to the boundary of the sides forwards to between the front legs, and backwards to the end of the wool between the hind legs. The points are represented by the lower woolled part of the limbs, about as far down as the imaginary line delimiting the sides and belly.

Twenty measurements were made on the right-hand side of each sheep while lying on a table: six in the neck region, eight on the back and sides and six on the belly and points. Of the last-mentioned, four were taken on the belly and one on each point. These are about the number employed in practice in estimating the length of a fleece, and may be deemed to give a fairly reliable average for the three areas.

TABLE 1.

Staple Length Averages on Live Sheep (cm.).

No.	Neck.	Body.	Belly.	No.	Neck.	Body.	Belly.
1	9.43	8.42	7.17	14	8.08	7.15	6.98
2	11.60	10.43	7.50	15	9.71	8.25	7.30
3	9.91	8.08	7.23	16	8.56	7.23	6.26
4	8.98	7.86	6.28	17	10.23	8.27	7.03
5	10.13	8.58	8.13	18	9.35	7.98	6.83
6	9.50	8.27	6.41	19	6.81	6.55	5.41
7	11.66	10.61	9.95	20	8.26	7.46	6.41
8	8.78	7.61	6.65	21	9.53	8.00	6.55
9	8.15	7.15	6.43	22	7.96	7.35	5.98
10	10.31	8.55	7.93	23	8.23	7.29	6.51
11	8.95	7.58	6.60	24	8.68	8.37	7.21
12	7.56	6.81	6.21	25	7.73	6.91	6.01
13	9.45	7.93	7.23				

	Neck.	Body.	Belly.
Mean.....	9.10 cm.	7.95 cm.	6.89 cm.
E. of M.....	$\pm .155$	$\pm .127$	$\pm .118$
S.D.....	1.152	.942	.876
C. of V.....	12.6 %	11.8 %	12.7 %
Ratios.....	100	87	76

Correlations: Neck and Body, $+.94 \pm .017$; Neck and Belly, $+.83 \pm .043$; Body and Belly, $+.84 \pm .039$.

Attention may be drawn to the fact that the measurements were all made on unshorn lambs, in contrast with those on woolled skins of uncertain history. Results based on sheep after one or more shearings are not likely to be reliable, for in practice a shearer leaves more wool on some parts of the body than on others, which will influence the later staple length.

The averages of the measurements in each of the three areas are given in Table 1 for each of the twenty-five sheep. In every case it will be seen that the neck staples are the longest, those of the body come next, while those of the belly are the shortest. The actual lengths vary much in the different sheep, as might be expected in a mixed flock; but the co-efficient of variability is least for the body, 11.8 per cent., as compared with 12.6 per cent. and 12.7 per cent. for the neck and belly respectively. The averages for all the sheep are 9.10 cm., 7.95 cm. and 6.89 cm. respectively, that is, the neck wool is about a centimetre longer than the body wool, and slightly more than two centimetres longer than the belly wool. If the neck wool be represented by 100 that of the body would be 87 and of the belly 76, ratios which compare very closely with those already given for the woolled skins, namely, 100, 89 and 76. In both series the neck covering is about 12 per cent. longer than that of the body, and about 25 per cent., or a quarter, longer than that of the belly. A high correlation holds between the staple length of the neck and body areas, and between the neck and belly, showing that one varies almost directly as the other, that is, a fleece long or short in one part will be correspondingly long or short elsewhere.

COMPARISON OF LENGTH DISTRIBUTION AND OF QUALITY (THICKNESS) DISTRIBUTION.

As previously remarked, a study has been made of the quality (thickness) variation and distribution within the merino fleece (1931). It now becomes of some interest to compare the staple length variation and distribution with that of the quality variation and distribution. It will be seen that the two, length and thickness, do not necessarily represent corresponding values.

The staple length has been found to diminish in a regular gradient manner from the neck region to the hindmost and under parts of the body. The highest quality wool on the other hand, that is, the finest and most desirable, occurs over the shoulder area, which in length is usually intermediate between that of the neck and the body. The neck wool is invariably stronger (thicker) than that of the shoulder and usually than that of the body.

Further, the wool towards the hind end of the body and over the britch and belly is the shortest, and at the same time is the thickest or lowest in quality.

The neck-length wool, here found to be the longest, is therefore neither the strongest nor the finest wool of the fleece; the intermediate length-wool of the shoulder area is the finest; the shortest wool at the hind region is the coarsest wool.

In a previous paper (1931) it has been shown that, as far as any single staple is concerned, the length of the fibres varies directly as the thickness; the thickest fibres are the longest and the finest are the shortest. Manifestly the above results show that the correlation does not hold when the wool of one area is compared with that of a distant area, for in the neck occurs the longest wool and at the hind end of the body the shortest wool, yet the former is always finer than the latter; the shoulder wool is by no means the shortest, and yet it is almost invariably the finest.

SUMMARY.

1. The variability in length of the fleece of the merino sheep, and its distribution over the different parts of the body, are studied, based on staple measurements of woolled skins and of the live animal.

2. No sharp line of separation can be drawn between the staple lengths over adjacent areas, but a gradient exists from before, backwards and downwards. On the average the wool covering the neck and shoulders is the longest, that over the back and sides the next longest, and that over the belly, points and britch the shortest. A fleece long or short in one part will be correspondingly long or short in other parts.

3. On the woolled skins the neck wool on the average is found to be 11 per cent. longer than the body wool, and 24 per cent. longer than the belly and britch wool, that is, if the length of the neck wool be represented by 100, that of the body and sides will be represented by 89 and that of the belly, points and britch by 76.

4. On the live sheep the neck wool is found to be 12 per cent. longer than the body wool, and 25 per cent. longer than the belly and points wool, that is, if the length of the neck wool be represented by 100, that of the body and sides will be represented by 87 and that of the belly and points by 76.

5. In both series of measurements the neck covering is about 12 per cent. longer than that of the body and 25 per cent., or a quarter, longer than that of the belly.

6. The distribution of the measurements reveals that the wool covering the body and sides of the sheep represents 58 per cent. of the whole area of the unskirted fleece; that covering the neck is 22 per cent. of the whole; while that over the belly, points and britch is 20 per cent. Approximately the merino fleece may be regarded as comprising one-half body-length wool, and one-quarter neck-length wool and another quarter belly and britch wool.

7. The staple length distribution in the fleece does not closely correspond with the quality or thickness distribution. Thus the neck-length wool, found to be the longest, is neither the strongest nor the finest wool of the fleece; the intermediate-length wool of the shoulder area is the finest; the shortest wool at the hind region is the coarsest wool.

8. So far as any single staple is concerned a direct correlation exists between the length and thickness of the individual fibres, the longest fibres are the thickest and the shortest are the finest; but no correlation holds when the wools from distant areas of a sheep are compared. Thus the wool is the longest in the neck region and the shortest towards the hind region, yet the former is always finer than the latter; the shoulder wool is by no means the shortest, and yet it is the finest of the fleece.

9. The ideal sheep would be one in which the fleece has the same staple length all over, and the selective breeding at present carried on should tend to encourage this.

REFERENCES.

- DUERDEN, J. E., and BELL, D. G. (1931). "Quality Variation and Distribution in the Fleece of the Merino." *17th Rept. of the Dir. Vet. Serv. and Animal Ind., U. of S.A.*, Pt. 2, pp. 789-806.

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Section IX.

Dips and Dipping.

- H. GRAF AND T. J. WILKEN-JORDEN. Researches into Dips and Dipping. A. Lime-Sulphur Dips. Paper I. General Introduction. Lime Sulphur Dips.
- T. J. WILKEN-JORDEN. Researches into Dips and Dipping. A. Lime-Sulphur Dips. Paper II. A New Laboratory Method of Chemical Analysis.
- T. J. WILKEN-JORDEN. Researches into Dips and Dipping. A. Lime-Sulphur Dips. Paper III. A Preliminary study of a Colorimetric Method as a Rapid Means of Control of Polysulphide Solutions.

A.—Lime-Sulphur Dips.

Paper I: General Introduction. Lime-Sulphur Dips.

By H. GRAF, B.Sc., B.V.Sc., Veterinary Research Officer, and
T. J. WILKEN-JORDEN, D.Sc., Dip Research Chemist,
Onderstepoort.*

THE use of various chemical substances in relatively dilute solutions for the destruction of external parasites on domestic stock plays an ever increasing rôle in veterinary science and this method of preventive veterinary medicine is yielding excellent results in the control and combating of many stock diseases as, for instance, in the case of tick-transmitted diseases such as East Coast fever, anaplasmosis, red-water, heartwater of cattle and sheep and nuttalliosis of horses.

The value of the incidental removal of parasites which produce a lowering of vitality and resistance to disease owing to their blood-sucking activities and the resultant annoyance and irritation of the host caused by their attacks on the skin or their penetration into the subcutis, cannot easily be over-estimated. The damage caused by the scab parasite, *Psoroptes communis* var. *oris*, at one time threatened the very existence of the wool farming industry in South Africa, but due to the efficacious dipping methods the damage has been practically eliminated and its complete eradication would appear to be only a matter of time. In spite of the excellent results obtained by means of dipping in the control of various diseases, the number of different chemical substances used as parasiticides is relatively small and their use is not in all cases free from danger. We need merely mention the occurrence of severe scalding of stock under certain conditions after immersion in arsenical fluids, the temporary reduction in the milk yield of dipped cows, the danger of fatalities due to the accidental ingestion or aspiration of dipping fluids, the possible injury to the hide or wool by frequent immersions, the heavy losses occasionally encountered especially in sheep and goats after dipping in so-called "phenolic" or "tar product" dips. In actual practice the control of dipping fluids, both as regards the concentration of the effective ingredient or ingredients and their possible conversion into less active substances either through atmospheric oxidation, contamination or micro-organic activities are also problems not yet satisfactorily solved in many cases. The exact mechanism of the absorption of dipping fluids by the parasite and how the toxic substances, once introduced, destroys the parasites is in practically all cases mere conjecture. Data on these aspects may prove particularly valuable from a practical point of view in perhaps indicating even more efficacious methods of attack, either in the methods of application of the dip to the parasite or indicating other groups of substances preferable either on the score of economy, efficacy or safety.

* This work has been carried out with the aid of a grant from the Empire Marketing Board.

Owing to the generosity of the Empire Marketing Board, funds became available for researches into various problems associated with dipping. A comprehensive scheme for research has been drawn up and the present series of papers are the initial results of the work undertaken.

LIME-SULPHUR DIPS.

In 1915 Shilston, working at the Allerton Veterinary Research Laboratory, Pietermaritzburg, published his observations on the scab parasite *Psoroptes communis*, var. *ovis*, dealing with the subject under the following headings:—

- (a) The life-history of the *Psoroptes communis*, var. *ovis*, on the sheep.
- (b) The interval to be allowed between dippings.
- (c) The duration of vitality of acari and their eggs apart from sheep; infectivity of kraals, etc.
- (d) The possibility of the *Psoroptes communis*, var. *ovis*, maintaining its existence on animals other than sheep, or of the *psoroptes* of such animals producing scab in sheep.
- (e) Variations in the rapidity of multiplication of the acari.

In the same report Bedford (1915) makes a further descriptive contribution concerning his observations on *Psoroptes communis* at Onderstepoort carried out at all seasons of the year on both short and long woolled sheep.

Resorting to dipping as a means of eradicating scab, theory would demand two successive immersions within the life-cycle of the scab parasite, the first immersion destroying the live acari as such, and the second immersion serving to kill off acari subsequently hatched from uninjured eggs without, however, allowing sufficient time for ovigerous females to develop and lay new eggs. From the work by Shilston and Bedford already quoted the optimum interval between successive dippings was found to be nine days. That the ova of *Psoroptes communis*, var. *ovis*, are resistant to the various dipping fluids was shown by Bedford (1928) by dipping the ova *in vitro* for various periods and afterwards replacing them on sheep.

At the request of the Sheep Division of the Union eighteen different proprietary and home-made sheep dips in use in South Africa were tested out (Bedford, 1915) on an experimental scale at Onderstepoort, the dips tested being:—

(1) Home-made Lime-sulphur dip, (2) Capex Lime-sulphur Concentrate, (3) Madderfontein Lime-sulphur Concentrate, (4) Home-made caustic soda and sulphur dip, (5) O'Gorman's Liquid Sulphur Dip, (6) Home-made Loogas-Sulphur Dip, (7) Little's Fluid Dip, (8) Hayward's Paste Dip, (9) McDougall's Powder Dip, (10) Cooper's Sheep Dipping Powder, (11) Arsenite of Soda and Sulphur Dip, (12) Jeye's Fluid, (13) Kerol, (14) Leach's Sheep Dip, (15) McDougall's Tobacco Dip, (16) Dreadnought Tobacco Dip, (17) Magic Sheep Dip, and (18) Home-made Tobacco Dip. The home-made dips were prepared according to the formulae given in

Bulletin No. 3, Department of Agriculture, Union of South Africa, while the proprietary dips were diluted according to specifications given by the manufacturers. With two-minute immersions and two successive dippings at an interval of nine days all these dips were found to be efficacious in eradicating scab. The possible exception in the case of O'Gorman's dip must be ascribed to the use of excessive dilution.

Having established the efficacy of dipping as a means of controlling scab, there still remained the possibility of re-infection from kraals and other enclosures into which dipped or clean sheep were admitted. The controversy centring around the possible infectivity of kraals seems to have been finally settled by exhaustive experiments conducted and reported on by Du Toit (1932). These experiments showed conclusively that infected kraals become perfectly free from infection after a period of seventeen days. Moreover, even infected kraals appear to be safe provided the sheep are dipped properly.

Regarding the regulation of anti-scab dips in the Union, the following historical development appears rather interesting. Under the Scab Regulations No. 1703 of 1919 an "authorized dip" was described as "a lime and sulphur dip prepared by the inspector". In Government Notice No. 1034 of July, 1921, an "authorized" dip was defined as a home-made lime and sulphur dip or any manufactured lime and sulphur dip which, when diluted, shall contain not less than 1.5 per cent. of polysulphide sulphur. Polysulphide sulphur was defined, in terms of Government Notice No. 1717 of October, 1922, as sulphur in the form of calcium pentasulphide or of calcium polysulphides not below the tetrasulphide. Under the list of "authorized" dips were also indicated tobacco dips made from tobacco grown in South Africa, the tank fluid containing not less than 0.05 per cent. and not more than 0.07 per cent. nicotine.

In Government Notice No. 1782 of October, 1925, dip was defined as an effective scab-destroying liquid of sufficient strength to ensure the destruction of the mite and registered under the regulations of Act No. 21 of 1917 (Fertilizers, Farm Foods, Seeds and Pest Remedies Act). For this purpose certain dips were approved of, an "approved" dip being defined as a dip the use of which has been sanctioned by the Minister of Agriculture for the dipping of infected sheep under official supervision, and which has been notified by him by notice in the *Government Gazette* as an approved dip. Such a list of approved dips were laid down in Government Notice No. 1783 of October, 1925, the dips approved of being:—

- A. Home-made lime-sulphur prepared according to recommendations of the Department of Agriculture, and commercial lime-sulphur or soda-sulphur concentrates, sold under directions such that when diluted for use the tank strength corresponds to not less than 1.5 per cent. "sulphide sulphur". Under this class the following registered dips were grouped:—

Capex Sulphur-lime Sheep Dip.

Kynoch and McDougall's Lime-sulphur Dip.

Little's Concentrated Sulphur Dip.

- B. Tobacco extracts and nicotine dips sold under directions such that when diluted for use the tank strength corresponds to not less than 0.05 nicotine. Under this class the following registered dips were grouped:—

Capex Nicotine Sulphate.
 McDougall's Lion Brand Tobacco Extract.
 Eagle Brand Tobacco Extract.
 Delmore Tobacco Dip.
 Delmore Tobacco Soap Dip.

- C. Arsenic-sulphur dips considered individually by the Department of Agriculture. These include:—

Cooper's Sheep Dipping Powder.
 Little's Powder Sheep Dip.

- D. Phenolic dips and tar distillates considered individually by the Department of Agriculture. These include:—

Little's Chemical Fluid Sheep Dip.
 McDougall's Sheep Dip (paste).
 Hycol No. 2 Steekhard Brand.

- E. Preparations containing other active ingredients, considered individually by the Department. These include:—

Kynoch's and McDougall's "Kynac."
 McDougall's Non-poisonous Powder Dip for Sheep.

This list has been slightly amended by Government Notice No. 2351 of December, 1925, Little's Concentrated Sulphur Dip being omitted under Class A; under Class B it was specified that the nicotine dips had to be manufactured in South Africa from tobacco grown in South Africa.

Since March, 1929 (Government Notice No. 438), only lime-sulphur or soda-sulphur dips are considered as "approved" dips for combating scab. Commercial concentrates are required to be sold under directions for use prescribing a tank strength which corresponds to not less than 1.5 per cent. "sulphide sulphur".

Proprietary lime-sulphur concentrates are thus generally sold on a guarantee-basis of 39 grams polysulphide sulphur per 100 c.c. concentrate, it being prescribed to dilute these concentrates to the extent of 1 in 25. This would yield a dipping fluid containing approximately 1.5 per cent. polysulphide sulphur. It would appear that dilutions down to about 0.8 polysulphide sulphur still produces an effective dipping fluid. To what extent, however, such dilution may be carried is not known, since the minimum effective concentration has not been determined.

On the other hand it is the practice in this country frequently to resort to home-made lime-sulphur dips. Such dips are prepared by boiling up 25 lb. sulphur and 20 lb. slaked lime (or 15 lb. unslaked lime) with 30 gallons water for 30-40 minutes, and after sedimentation diluting the concentrate to 100 gallons. Assuming it were possible to bring all the sulphur into solution in this way, the concentration of total sulphur in solution would be 2.5 per cent. (vol.). Of this total sulphur about 6/7 or 2.1 per cent. will be polysulphide sulphur,

the rest being thiosulphate sulphur. Taking the calcium polysulphides in solution to be represented by the average empirical formula $\text{CaS}_{1.6}$, the theoretical amount of lime necessary to give a solution of 2.1 per cent. polysulphide sulphur amounts to ± 12 gm. chemically pure calcium hydroxide for every 1,000 c.c. solution. On the basis of using 20 lb. lime for every 100 gallons dip fluid it would mean that a commercial lime containing +60 per cent. active lime (dip coefficient = ± 60 per cent.) should still yield a theoretical polysulphide sulphur concentration of 2.1 per cent. On this basis a very poor lime of only ± 25 per cent. purity should theoretically still produce an effective dipping fluid. In the actual practice of home-made dips, however, the dipping fluids prepared from such inferior limes apparently invariably fail to come up to strength, although dip samples prepared in the laboratory usually closely approximate the theoretical value. (Green, 1915c.)

In order to explain this discrepancy, and also to answer various other questions of direct practical importance to the farmer, we have undertaken a rather extensive survey of the application of home-made lime-sulphur dips in the field, obtaining samples and detailed information from all parts of the Union. For the purpose of this survey twelve sets of containers were despatched to the Government Veterinary Officers of each of the five Provinces; Transvaal, Free State, Cape, Transkei and Natal; each set of containers being accompanied by a questionnaire asking for the information required. To avoid further unnecessary explanation the questionnaire is here reproduced:—

LIME-SULPHUR QUESTIONNAIRE.

IMPORTANT.—This investigation will have no real value, unless all officers concerned assist in giving the required information as accurately as possible.

Bottles and tins, bearing typed labels, are supplied for the necessary samples.

Containers and purpose specified.

It is urgently requested that officers shall study the labels thoroughly, and that these be adequately, accurately, and fully filled in. To exclude air, the bottles must be thoroughly filled right up to the cork, well-stoppered, and the full set forwarded immediately to the Director of Veterinary Services, Pretoria North Station.

1. *Preparation of Dip-concentrate.* Please state:—

- (a) District..... Farm..... Owner.....
- (b) Weight lime used.....
- (c) Origin of lime and brand.....
- (d) Weight of sulphur used.....
- (e) Brand of sulphur used.....
- (f) Quantity of water used.....
- (g) Particulars concerning the boiling up, settling and separation of liquid from sediment.....
- (h) Date on which concentrate was made.....

Please send samples of—

- (a) lime used—bottle marked (1);
- (b) water used—bottle marked (2);
- (c) freshly-prepared concentrate—bottle marked (3).

2. *Dilution of concentrate down to Dipping Strength.*

Please state:—

- (a) The dilution, i.e. to what volume (in gallons) the *total* concentrate was diluted.....
- (b) Particulars concerning dipping tank approx. capacity.....
- (c) Date on which concentrate was diluted.....

Please send samples of—

- (a) The diluted dip fluid as prepared for immediate use—bottle marked (4).

N.B.—When diluting the concentrate the liquid must be thoroughly stirred before taking the sample, so that the sample may represent the total contents of the tank.

3. *Dip Fluid after First Dipping.*

Please state:—

- (a) Number of sheep (goats) dipped.....
- (b) The time (in hours) required to dip the specified number of animals.....
- (c) Date(s) of dipping.....

Please send samples of—

- (a) Dip fluid *immediately after* 1st dipping—bottle marked (5).

4. *Dip Fluid for 2nd Dipping.*

Please state:—

Whether the same dip fluid was used again for the 2nd dipping.....
 If *yes*, please state following:—

- (a) Date of 2nd dipping.....
- (b) Number of sheep (goats) dipped during 2nd dipping.....
- (c) Time (in hours) required to dip the specified number of animals.....

Please send samples of—

- (a) Dip fluid *immediately before* 2nd dipping—bottle marked (6);
- (b) Dip fluid *immediately after* 2nd dipping—bottle marked (7).

N.B.—In case it was considered necessary to strengthen the dip fluid before use by adding some freshly made concentrate, please forward samples of concentrate added, of the unfortified dip fluid, and of the fortified fluid *before* and *after* dipping—bottles marked (8).

Also state the number of gallons concentrate used for 100 gallons fluid.....

General Information.

- (a) When was scab last observed on the farm?.....
- (b) Was any peculiarity observed when dipping goats in lime-sulphur dip?.....
 If so, briefly describe phenomena observed, stating number of goats dipped and how many of these were affected.....
- (c) What is your opinion regarding the effectiveness of lime-sulphur as a dip against scab?.....



In the accompanying sketch of the Union of South Africa the different districts included in the survey have been marked off in outline, and in the table data have been tabulated relating to the number of sheep and goats in each district, the wool production, and the outbreaks of scab in those districts during the last two years. The wool production figures relate to the statistical year 1928-1929. In connection with the figures given for scab outbreaks, these figures represent the percentage of infected flocks over the years 1930 and 1931, the number of flocks being given in terms of the amended definition of a flock as laid down in Government Notice No. 278 of 14th February, 1930.

Apart from this survey dipping experiments are in progress to determine the minimum effective polysulphide concentration. Such information is considered essential since, on the one hand the uncertain and frequently poor quality of the lime often results in a dip-wash appreciably below the desired strength and on the other hand unnecessarily high polysulphide concentrations would naturally involve risk of damage to the wool and probably render it less amenable to scouring and cleaning processes in general. In this connection the need of a rapid and effective field method of analytical control of dip-washes becomes self-evident.

Coming now to the chemical reactions involved in the preparation, handling and use of polysulphide dips the evidence at hand seems to indicate that this, in reality the most important, aspect of dipping problems has in this country been sadly neglected by those interested only in the practical application of such dips. In this connection the studies of H. H. Green (1915 A and B) on the chemistry of polysulphide dips must be classed as a notable exception. It is general knowledge that in the course of dipping in polysulphide solutions the dip decreases in strength (Van Zyl, 1926), sulphur being precipitated and thiosulphate being formed. As far as we know, no quantitative study of the reactions involved has been made. These reactions are now being studied by studying quantitatively the action of atmospheric oxygen and carbon dioxide upon polysulphides in solution. The production of thiosulphate as final air-oxidation product is certainly strange when considered in the light of modern knowledge regarding the chemical mechanism of oxidation reactions. In this connection it must be remembered that, after immersion of the animal in the dipping tank, the dip dries on the body in contact with the atmosphere. It is considered to be of the greatest importance to know exactly what happens to the polysulphide on the skin and wool immediately after the animal leaves the dipping tank, since it has been shown by various workers that the real toxic action of sulphur and sulphur preparations must be ascribed to the presence of higher polythionic acids. It therefore becomes apparent that, what would at first sight appear to be a purely academical investigation into the mechanism of the oxidation of polysulphide solutions, becomes a question of direct practical and perhaps economical importance.

The question of combating and controlling scab on wool-bearing sheep is not merely a question of destroying the responsible parasite, but also the eradication of the parasite without the slightest dele-

terious effect on the wool produced. This question is of such vital importance to the Union that it has been decided, in spite of the favourable report by Hollis, to re-investigate the possible effects of dips on the wool, this time concentrating on the chemical effect of such dips on the wool fibre.

Apart from this general broad outline there are of course various other minor aspects which deserve attention. These, however, we consider better suited for discussion in the more specialized papers following later in the series.

SUMMARY.

A general introduction to the subject of dips and dipping is given, followed by a discussion of the past and present use of lime-sulphur dips in South Africa as a means of combating sheep scab. A programme of research into the various phases of lime-sulphur dips has been drawn up and reproduced here in its broadest outline.

LITERATURE.

- BEDFORD, G. A. H. (1915). Experiments and observations carried out with *Psoroptes communis* at Onderstepoort. *3rd & 4th Repts. of the Dir. Vet. Res. U. of S.A.*, p. 99.
- BEDFORD, G. A. H. (1915). Report upon the Dipping Trials carried out with the different Proprietary and Home-made Sheep Dips in South Africa. *3rd & 4th Repts. of the Dir. Vet. Res. U. of S.A.*, p. 163.
- BEDFORD, G. A. H. (1928). The Effect of various Dipping Fluids upon the Oviparous Females of *Psoroptes communis* var. *ovis*, and their Ova. *13th & 14th Repts. of the Dir. Vet. Educ. & Res. U. of S.A.*, pt II., p. 859.
- DU TOIT, P. J. (1923). Sheep Scab: The Infectivity of Kraals. *9th & 10th Rept. of the Dir. Vet. Educ. & Res. U. of S.A.*, p. 221.
- GREEN, H. H. (1915a). The Sulphur Sheep Dips. *3rd & 4th Rept. of the Dir. Vet. Res. U. of S.A.*, p. 113.
- GREEN, H. H. (1915b). Upon the Composition and Analysis of Poly-sulphide Solutions. *3rd & 4th Repts. of the Dir. Vet. Res. U. of S.A.*, p. 173.
- GREEN, H. H. (1915c). The Sulphur Sheep Dips. *3rd & 4th Rept. of the Dir. Vet. Res. U. of S.A.*, p. 141.
- SHILSTON, A. W. (1915). Observations on the life-history of *Psoroptes communis*, var. *Ovis*, and some points connected with the epizootiology of the disease in South Africa. *3rd & 4th Repts. of the Dir. Vet. Res. U. of S.A.*, p. 69.
- VAN ZYL, J. P. (1926). Notes on the Decomposition of Diluted Polysulphide Dips. *11th & 12th Repts. of the Dir. Vet. Educ. & Res. U. of S.A.*, p. 139.

Dis- trict No.	District.	Total Flocks, Sheep.	Woolled Sheep.	Non- woolled Sheep.	Total Flocks, Goats.	Angora Goats.	Other Goats.	Total Stock in District.	1928-29 Wool Production.		Flock % Scab Outbreaks.			
									Wool.	Mohair.	1930.		1931.	
											Sheep.	Goats.	Sheep.	Goats.
1	Piet Retief.....	189	92,471	2,459	208	—	40,808	135,708	Bb.	Bb.	3.7%	1.4%	6.3%	—
2	Ermedo.....	1,230	672,552	3,536	303	111	16,169	632,368	477,835	149	0.9%	0.6%	0.2%	—
3	Barberton.....	167	13,001	8,108	226	—	47,368	88,477	214,291	—	1.8%	—	3.0%	—
4	Pietersburg.....	979	38,524	114,068	1,010	482	272,826	425,940	200,650	972	1.5%	0.3%	0.9%	—
5	Potgietersrus.....	465	2,002	37,857	908	—	36,282	76,141	12,346	—	1.1%	—	0.3%	—
6	Middelburg (Tvl.)	1,170	180,585	31,201	1,710	911	73,820	286,517	1,014,356	386	1.3%	0.1%	1.4%	0.1%
7	Pretoria.....	2,308	54,945	72,637	2,418	—	74,370	201,952	322,363	75	0.2%	0.1%	0.3%	—
8	Vryburg.....	3,757	196,844	116,661	2,833	5,831	200,546	512,942	692,807	2,867	0.7%	0.2%	0.1%	0.3%
9	Kuruman.....	1,320	297,582	150,533	1,231	1,152	168,598	617,865	1,370,321	5,923	0.1%	4.3%	0.1%	2.4%
10	Kimberley.....	221	24,210	49,234	196	—	21,755	95,167	143,338	220	0.5%	—	—	—
11	Hay.....	794	207,645	393,491	792	10,416	173,809	785,361	928,479	37,558	0.8%	1.6%	0.1%	1.5%
12	Kenhardt.....	1,025	361,462	256,605	615	3,078	124,374	745,519	850,092	16,900	8.8%	1.9%	2.7%	1.1%
13	Namaqualand.....	514	104,862	216,053	528	—	235,903	556,818	230,712	—	9.3%	1.3%	8.7%	1.5%
14	Calvinia.....	985	477,812	214,396	523	—	117,635	809,843	1,502,136	1,304	3.1%	0.8%	1.2%	1.5%
15	Carnarvon.....	559	447,208	130,490	345	3,479	54,251	635,428	1,085,379	14,328	7.9%	0.9%	1.4%	2.0%
16	Beaufort West.....	746	480,952	127,814	311	55,901	37,800	702,467	1,976,708	148,241	1.7%	0.6%	1.1%	1.6%
17	Graaff-Reinet.....	345	372,088	79,184	227	27,146	30,412	508,830	2,742,911	102,459	—	1.9%	1.9%	1.9%
18	Kingwilliamstown	430	419,572	760	227	—	132,281	552,613	1,817,249	—	7.0%	0.4%	8.4%	0.4%
19	Queenstown.....	1,257	536,271	6,612	699	10,068	14,978	567,923	3,273,588	30,565	—	—	—	—
20	Butterworth.....	42	126,749	—	27	—	27,801	154,550	596,463	—	—	11%	2.4%	7.4%
21	Umtata.....	131	278,417	—	121	—	41,455	319,872	1,091,771	—	—	5.0%	5.8%	5.8%
22	Port St. Johns.....	19	23,165	—	20	—	22,978	46,143	67,363	—	—	20%	—	15%
23	—	—	—	—	—	—	—	—	—	—	—	—	—	—
24	Port Shepstone.....	70	582	4,630	116	77	16,146	21,435	3,917	—	—	—	2.8%	—
25	Pietermaritzburg.....	55	1,583	3,940	61	4	8,960	14,487	1,645	—	—	—	1.8%	1.6%
26	Edenburg.....	351	182,338	15,943	282	1,584	46,477	246,342	1,070,174	5,779	—	—	0.3%	—
27	Eshowe.....	37	—	13,759	35	—	53,677	67,436	—	—	—	—	2.7%	—
28	Vryheid.....	479	181,140	8,810	751	160	50,318	240,428	1,734,394	1,242	19%	—	—	—

* Scab outbreak figures for Waterberg included.

Researches into Dips and Dipping.

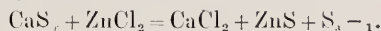
A. Lime-Sulphur Dips.

Paper II. A New Laboratory Method of Chemical Analysis.*

By T. J. WILKEN-JORDEN, D.Sc., Dip Research Chemist,
Onderstepoort.

THE last report on lime-sulphur dips issued by this Institute was made by H. H. Green in a contribution "Upon the Composition and Analysis of Polysulphide Solutions", published in the Third and Fourth Reports of the Director of Veterinary Research, November, 1915. In that report Green contributes a brief but valuable discussion of the analytical methods in use up to about 1915.

According to the old A.O.A.C. methods previous to 1911 the "monosulphur equivalent" or base present in combination in sulphide or polysulphide form is determined titrimetrically with N/10 ammoniacal zinc chloride, using nickel sulphate as external indicator:—

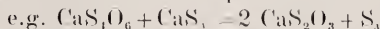


The thiosulphate is then determined iodometrically in the filtrate.

Harris (1911) suggested the titration of polysulphide solutions with iodine until the disappearance of the yellow colour. This procedure necessarily assumes that, in the presence of thiosulphate, the iodine reacts selectively and quantitatively with the sulphide or sulphide equivalent present until all sulphide has been removed. This assumption, at least in its practical effect upon the final result obtained, has been experimentally confirmed by the present author. Considered superficially, such an assumption appears to be at variance with general theory. However, on considering the reactions involved more exhaustively, we find that this assumption is in accordance with known chemical facts. Any iodine in local excess of the amount of sulphide present during the course of the titration will naturally react with the thiosulphate according to the well-known equation—



However, if any sulphide (or polysulphide) still be present in the solution, the reaction does not end here. It has been shown by various workers [Chancel and Diacon (1863), Smith and Takamatsu (1882) and Kurtenacker and Kaufmann (1925)], that the polythionates readily react with sulphides or polysulphides, regenerating the equivalent amount of thiosulphate—



* This work has been carried out with the aid of a grant from the Empire Marketing Board.

As far as the final reaction state is concerned it is therefore immaterial whether the sulphide is directly titrated with iodine or whether an equivalent amount of thiosulphate is first converted into tetrathionate by iodine titration and the tetrathionate then added to the sulphide solution. In fact, as has been suggested by Chapin (1916a), sulphide solutions can readily be titrated with a standard solution of tetrathionate.

In 1916 the A.O.A.C. (1916) advocated as official method the precipitation of polysulphide sulphur by ammoniacal zinc chloride as ($\text{ZnS} + \text{S}_{x-1}$), filtering off, and determining this sulphur gravimetrically as barium sulphate. The thiosulphate is determined iodometrically in the filtrate. This method has also been retained in the 1920 publication of the A.O.A.C. (1920). In the latter report the substitution of the ammoniacal zinc chloride by an ammoniacal cadmium chloride solution was also tested out. Needless to say, the cadmium chloride method presented difficulties on account of the extreme difficulty of affecting complete oxidation of cadmium sulphide in alkaline solution.

Of greater practical importance are the studies by Chapin (1916b) on the volumetric analysis of polysulphide solutions. Chapin determines the monosulphide sulphur equivalent by titrating with tetrathionate, while the free polysulphide sulphur (S_{x-1}) is determined by allowing the polysulphide solution to run into an ammoniacal zinc chloride solution, adding excess sodium sulphite, heating till all free sulphur is dissolved, precipitating excess sulphite with strontium chloride, and filtering. In this way the free polysulphide sulphur is titrated iodometrically as thiosulphate.

Apart from these methods, pure science has not been wanting in its contribution. Bodnár, in 1914-15, applied the argentometric method to the analysis of polysulphide solutions. A measured quantity of sulphide solution is pipetted into an excess of N/10 silver nitrate solution, and the precipitate of silver sulphide and sulphur filtered off. The thiosulphate is determined by titrating the free sulphuric acid formed according to the equation—



The thiosulphate plus monosulphide equivalent is determined by titrating the excess silver in the filtrate with N/10 ammonium sulphocyanate. This method has been checked by the writer and found to yield excellent results. Unfortunately, however, its general application is rather restricted, since it becomes inapplicable when (i) excess free base or acid is present, or (ii) when chlorides, cyanides, etc., are present.

About simultaneously with Bodnár's argentometric method Sander (1915) developed a mercurimetric method using a solution of mercuric chloride as reagent. Sander determines the thiosulphate plus sulphide by iodometric titration, and in a second aliquot determines the thiosulphate by adding excess mercuric chloride, filtering, and titrating the free hydrochloric acid in the filtrate formed according to the following equation:—



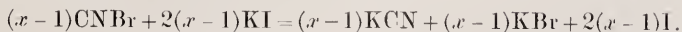
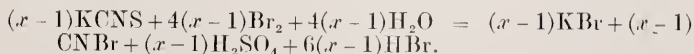
Both these methods are dependent on the introduction of an empirical factor in order to determine the polysulphide sulphur actually present. To overcome this disadvantage, Wöber (1917) has advocated the determination of thiosulphate and monosulphide equivalent according to the methods of Boduár or Sander, the free polysulphide sulphur (S_{-1}) being determined oxalkalimetrically. For this purpose Wöber pipettes the polysulphide solution into a known quantity of standard alkali solution, oxidizes to sulphate with hydrogen peroxide, and titrates the excess of free alkali remaining after completion of oxidation. This method has also been tried out by the writer, but has been found to give irregularly high results due to the action of the alkali on the glass and carbon dioxide contamination.

Muller (1915) determines thiosulphate plus sulphide by iodometric titration, and the thiosulphate alone by acidifying with acetic acid, shaking under vacuum to remove sulphide as sulphuretted hydrogen, and again titrating with iodine.

For the determination of sulphide in depilatory solutions Atkin (1922) proposes the titration with a standard solution of ammoniacal zinc sulphate using lead acetate paper as external indicator.

Jones (1923) separates monosulphide sulphur from free polysulphide sulphur (residual) and from thiosulphate by passing a current of carbon dioxide through the solution, and absorbing the sulphuretted hydrogen produced in a solution containing sodium peroxide. He claims such a separation to be efficient, quantitative and accurate.

A more interesting method of analysis has been evolved by Schulek (1925). Excluding air from the apparatus, the polysulphide solution is boiled with free boracic acid and potassium cyanide, thereby liberating the monosulphide equivalent as sulphuretted hydrogen and converting the free polysulphide sulphur into thiocyanate, while the thiosulphate remains unaffected. The thiosulphate is then determined by the ordinary iodometric titration, while the thiocyanate in another aliquot is determined by adding excess bromine water, removing the excess bromine with phenol, adding excess potassium iodide, and titrating the liberated iodine. The reactions involved may best be represented as follows:—



For the analysis of solutions containing sulphides, sulphites, and thiosulphate Kurtenacker and Bittner (1924) take three aliquots and then proceed as follows: In the first aliquot the sum of sulphite, sulphide and thiosulphate is determined iodometrically by adding excess iodine, acidifying and titrating the excess iodine. In the

second aliquot the sum of sulphide and thiosulphate is determined by adding excess zinc acetate and formaldehyde, acidifying with acetic acid, adding excess iodine, and titrating back the excess iodine. In the third aliquot thiosulphate alone is determined by adding zinc acetate, filtering off sulphide, treating with formaldehyde to remove sulphite, acidifying and titrating with iodine. In a further communication (1925) by the same authors the polysulphide sulphur is determined by heating with sulphite, adding zinc acetate, and filtering. The filtrate is treated with formaldehyde, acidified with acetic acid, and titrated with iodine.

A slight modification of Chapin's method for determining polysulphide sulphur has been suggested by Goodwin and Martin (1925), omitting, *inter alia*, the addition of 1-2 c.c. sodium phosphate solution as recommended by Chapin.

An improved procedure of Kurtenacker's method for analysing solutions containing sulphides, sulphites and thiosulphates has been given by Wollak (1929) who at the same time suggests Kurtenacker's cyanide method (1921) as a control for thiosulphate.

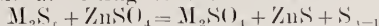
For determining the amount of polysulphide and thiosulphate in commercial sodium sulphide Shimo and Makita (1929) boil the sample with excess sodium sulphite and magnesium chloride in an atmosphere of carbon dioxide. The thiosulphate so formed by the action of the sulphite on the polysulphide is titrated with iodine, thereby converting the excess sulphite to sulphate and the thiosulphate to tetrathionate. The tetrathionate so formed is determined by reduction with metallic aluminium and hydrochloric acid, the evolved sulphuretted hydrogen being absorbed in excess iodine, and the excess iodine titrated.

The titrimetric determination of polysulphide sulphur by conversion to thiosulphate by means of sulphite is also recommended by Szeberényi (1929) who titrates the thiosulphate formed iodometrically in the presence of excess sodium bicarbonate. As a second method Szeberényi suggests the oxalkalimetric method originated by Wöber (1917).

With this brief review of the literature as introduction, we may now proceed to describe our volumetric cadmium acetate method. This method has been specially evolved to study the action of atmospheric oxygen and carbon dioxide on polysulphide solutions, and also to afford a somewhat exact and less laborious method of analysis in connection with a field survey of lime-sulphur dips at present in progress. A large number of analyses of actual lime-sulphur dips, both freshly prepared and highly adulterated through excessive dipping, have already been made, but so far we have found no serious difficulty in obtaining satisfactory results. We realize, however, that for the rapid laboratory control of dipping baths a high-speed method of analysis is highly desirable. With this object in view researches on analytical methods are still in progress.

DEVELOPMENT OF ANALYTICAL PROCEDURE.

The underlying principle of the volumetric Chapin zinc sulphate method for the determination of the free sulphur (Watson and Rajagopalan, 1922) liberated by the decomposition of the polysulphide according to the reaction:—



was found most serviceable for the determination of what we shall refer to in future as "free polysulphide sulphur"—F.P.S.S. By using a zinc salt as polysulphide reagent it is, however, impossible to determine the monosulphide sulphur equivalent—M.S.S.E.—since in excess ammonia some of the free sulphur is dissolved (Calcagni, 1921), and on filtration passes into the filtrate with the thiosulphate. Considering the fact that cadmium acetate has proved itself a valuable reagent for determining monosulphide sulphur, e.g. for the determination of H_2S in coal gas, it was decided to replace Chapin's zinc sulphate by cadmium acetate. The fact that cadmium sulphide is insoluble in dilute acetic acid, and that thiosulphate is not readily decomposed by very dilute solutions of this organic acid, made the use of an acid medium possible, thereby preventing the precipitation of calcium salts and avoiding the loss of free sulphur due to its solubility in alkaline media. By working in duplicate it, therefore, becomes possible to determine the M.S.S.E. in one fraction and the F.P.S.S. in the other.

It was found that under certain conditions—see detailed method below—the F.P.S.S. could be determined quantitatively by heating with excess sodium sulphite solution in an ammonialkaline solution, ammonium chloride being used as a catalyst to promote the reaction. Again, by adding excess N/10 iodine solution to the duplicate precipitate of $(\text{CdS} + \text{S}_{x-1})$, and then acidifying with concentrated hydrochloric acid and titrating back the excess iodine with N/10 thiosulphate solution, excellent values are obtained for the M.S.S.E. The thiosulphate is determined in the filtrate obtained by filtering off the precipitate of $(\text{CdS} + \text{S}_{x-1})$. In order to accelerate complete precipitation and avoid colloidal solutions of sulphur and cadmium sulphide, it was found necessary to add a strong electrolyte as coagulant. For this purpose a 10 per cent. solution of ammonium sulphate proved highly efficient, although ammonium chloride appears to function equally well.

In developing the analytical method it was found convenient to work with pure polysulphide solutions prepared by boiling fresh solutions of pure sodium sulphide with flowers of sulphur under exclusion of air. In this way the amount of polysulphide sulphur actually present could readily be controlled by the gravimetric determination of total sulphur as barium sulphate. From these polysulphide solutions synthetic dip fluids were prepared by adding known amounts of pure sodium thiosulphate—as N/10 solution. In the first part of this work the amount of thiosulphate in the duplicate filtrate was controlled argentometrically by the Bodnár silver nitrate method. Some of the results obtained in this way are tabulated in Table 1, the polysulphide solution used containing 1.364 gm. polysulphide sulphur (as total sulphur) per 100 c.c. solution, and representing the equivalent of 10.0 c.c. N/10 iodine per 5 c.c. solution for M.S.S.E.—as determined by direct titration with iodine—and the equivalent of 16.3 c.c. N/10 iodine for F.P.S.S.

TABLE 1.

Synthetical Solution.	M.S.S.E. c.c. N/10 I.	F.P.S.S. c.c. N/10 I.	Iodometric c.c. N/10 I.	Argentometric c.c. /2 N/10 AgNO ₃ .
(1) 5 c.c. polysulphide + 0.0 c.c. N/10 Na ₂ S ₂ O ₃ (f = 0.986)	10.0	16.3	0.1	Trace Ag ₂ S
(2) 5 c.c. polysulphide + 1.0 c.c. N/10 Na ₂ S ₂ O ₃ (f = 0.986)	9.95	16.4	0.87	0.77
(3) 5 c.c. polysulphide + 2.0 c.c. N/10 Na ₂ S ₂ O ₃ (f = 0.986)	10.05	16.3	1.74	1.58
(4) 5 c.c. polysulphide + 5.0 c.c. N/10 Na ₂ S ₂ O ₃ (f = 0.986)	10.0	16.3	4.40	4.40
(5) 5 c.c. polysulphide + 10.0 c.c. N/10 Na ₂ S ₂ O ₃ (f = 0.986)	9.95	16.3	9.15	9.27
(6) 5 c.c. polysulphide + 20.0 c.c. N/10 Na ₂ S ₂ O ₃ (f = 0.986)	10.05	16.4	18.8	19.2

From the table it is clear that the values obtained for the thio-sulphate are consistently slightly low,* the amount of percentage deviation from the theoretical value, however, systematically decreasing as the concentration of thiosulphate increases. This decrease in deviation is more clearly illustrated by the values tabulated in Table 2.

TABLE 2.

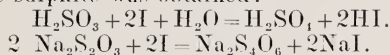
	Thiosulphate Added.	Thiosulphate Found.	Deviation.	Percentage Deviation.
(2).....	0.99 c.c. N/10	0.87 c.c. N/10	0.12 c.c. N/10	12.1
(3).....	1.97 "	1.74 "	0.23 "	11.7
(4).....	4.93 "	4.40 "	0.53 "	10.8
(5).....	9.86 "	9.15 "	0.71 "	7.2
(6).....	19.72 "	18.8 "	0.92 "	4.6

In order to apply this necessary correction to the thiosulphate values as determined, the theoretical values—in c.c., N/10 iodine—were plotted graphically against the determined values (see detailed method for graph), thus obtaining practically a straight line and affording a convenient means of affecting the necessary correction.

* This fact has been brought out by the work of Thompson & Whittier; Delaware College Agric. Expt. Sta. Bull. No. 105, p. 7 (1914) and reported by Chapin; Ind. Eng. Chem. 8, 339 (1916), the latter ascribing the low thio-sulphate values to absorption of thiosulphate by precipitated sulphur and sulphides.

Referring again to Table 1, it will be seen that, especially with the higher thiosulphate concentrations, the argentometric method yields values slightly higher than the amount of thiosulphate obtained by the ordinary iodometric titration. This seems to indicate that in the presence of cadmium acetate the thiosulphate suffers transformation to a certain extent, yielding a product of polythionic nature reacting with silver nitrate but not with iodine. As, however, the argentometric method, excellent as it is, is not applicable to limesulphur dips for reasons already mentioned, *inter alia*, on account of the invariable presence of varying amounts of chloride, resort was taken to the cyanide method of Kurténacker (Kurténacker and Fritsch, 1921; and Kurténacker and Goldbach, 1927).

This latter method of determining thiosulphates in admixture with sulphites or polythionates was found to yield most excellent results. Using solutions of pure sodium sulphite and sodium thiosulphate, mixtures were prepared of known thiosulphate and sulphite content. To aliquots of these solutions were added 5 c.c. of the standard cadmium acetate solution, diluted with water to about 150 c.c., 20 c.c. 20 per cent. acetic acid added, and titrated with N/10 iodine with starch as indicator. In this way the sum of thiosulphate plus sulphite was obtained:—



To the titrated solution containing the tetrathionate a few drops of phenolphthalein solution were added, and the solution then made distinctly alkaline with ammonia. To this ammoniakaline solution was added about 1 gm. of pure potassium cyanide, and the solution left to itself for half an hour. It was then acidified with 25 c.c. of 1:3 sulphuric acid and titrated immediately with N/10 iodine solution. The cyanide reacts with the tetrathionate forming thiosulphate according to the equation—



We observe that 1 mol. thiosulphate is formed for every 1 mol. of tetrathionate present, thus forming 1 mol. of thiosulphate for every 2 mols. originally present. The results so obtained have been tabulated in Table 3.

TABLE 3.

	Sulphite Present, c.c. N/10 I.	Thiosulphite Present, c.c. N/10 I.	Sulphite + Thiosulphate Found, c.c. N/10 I.	Thiosulphate Found, c.c. N/10 I x 2.	Sulphite Found, c.c. N/10 I.
(1).....	5.25	4.81	10.05	4.85	5.20
(2).....	5.85	9.62	15.55	9.60	5.95
(3).....	5.85	19.3	25.15	19.3	5.85
(4).....	5.25	28.86	34.19	28.98	5.20

Representing the direct iodine titration by A, and the titration after interaction of cyanide by B, the amount of sulphite, if present, is given by (A - 2B). For reasons which will be considered in later papers when discussing the chemical mechanism of polysulphide dips, the presence of sulphites in such solutions can be definitely excluded.

It is clear, however, that in cases where 2B exceeds A the difference is due to substances of polythionic nature as, e.g. tetrathionate. The cyanide method was therefore used to investigate the reactions responsible for causing low values when determining thiosulphate according to the cadmium acetate method.

To a mixture containing 10 c.c. of a 10 per cent. cadmium acetate [pure Cd (OOC.(CH₃)₂.2H₂O, free of sulphate] solution, 5 c.c. of a 20 per cent. acetic acid solution, and 20 c.c. of a 10 per cent. ammonium chloride solution, were added 10.6 c.c. N/10 thiosulphate. To each of these solutions in an Erlenmeyer flask was then added 5 c.c. of a sodium polysulphide solution containing 0.936 gm. polysulphide sulphur per 100 c.c. solution. A series of these mixtures, in duplicate, was allowed to react for different time intervals in a steam bath, one set being left to stand for 48 hours at room temperature. The general analytical procedure was to filter off the precipitate of (CdS+S_{x-1}), determining the M.S.S.E. in one precipitate and the F.P.S.S. in the duplicate precipitate. The one filtrate was acidified with 15 c.c. 20 per cent. acetic acid after dilution to about 150 c.c. and titrated with N/10 iodine (A. c.c.), then ammonia and potassium cyanide added, and later tritrated again with N/10 iodine (B c.c.) after acidification with sulphuric acid. The duplicate filtrate was titrated with iodine, then 20 c.c. N/1 hydrochloric acid added, and the sulphate present precipitated in the cold with 10 c.c. of a 10 per cent. barium chloride solution. The barium sulphate was filtered off after standing overnight. The results obtained have been tabulated in Table 4.

TABLE 4.
TITRATION READINGS.

Reaction Time.	A. Dir. Titration c.c. N/10 I.	B. with KCN c.c. N/10 I.	2B-A.	M.S.S.E. c.c. N/10 I.	F.P.S.S. c.c. N/10 I.
I.—0 hours.....	9.86	5.06	0.26	6.20	11.56
II.—1 hour.....	9.34	5.11	0.88	6.50	11.11
III.—2 hours.....	8.82	5.11	1.40	7.15	10.83
IV.—4 hours.....	7.55	4.98	2.41	8.83	10.04
V.—8 hours.....	4.67	4.28	3.89	12.60	9.38
VI.—48 hrs. (cold).	9.08	5.51	1.22	6.81	—

CALCULATED AS GRAMS S.

Reaction Time.	S ₂ O ₃ = S	S ₄ O ₆ = S	S= S	Sx-1 S	SO ₃ = S	Total S. calc.
I.—0 hours.....	.0631	.0617	.0099	.0370	.0000	0.1117
II.—1 hour.....	.0598	.0656	.0104	.0355	.0003	1.1116
III.—2 hours.....	.0564	.0690	.0114	.0347	.0005	0.1120
IV.—4 hours.....	.0483	.0154	.0141	.0321	.0011	0.1109
V.—8 hours.....	.0299	.0249	.0202	.0300	.0033	0.1083
VI.—48 hrs. (cold)	.0581	.0082	.0109	—	Trace	—

Tot. S. present = 0.0468 + 0.0678 = 0.1146 gm. S.

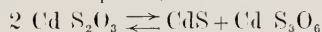
From these data it appears that the reaction is a time reaction, the thiosulphate sulphur T.S.S.—and F.P.S.S. decreasing with time, while the M.S.S.E. and the polythionic sulphur—P.T.S.—show a somewhat corresponding increase. In the above table the P.T.S., expressed as $(2B - A)$ c.c. N/10 iodine, has been calculated as tetrathionate sulphur. It is possible, however, that pentathionate is also formed since, according to Kurtenacker, both tetra- and pentathionic acids react with cyanide to form thiosulphate. In this reaction the first probable step is the formation of cadmium thiosulphate from the cadmium acetate and sodium thiosulphate present. According to Fock and Klüss (1890) cadmium thiosulphate decomposes according to the equation—



Under the present conditions of experiment this reaction, for reasons which are self-evident, is totally inapplicable, except perhaps in cases of extremely high dilutions with very low thiosulphate concentrations. The amounts of sulphate sulphur found are so minute that they may safely be attributed to atmospheric oxidation, since the reaction took place in open Erlenmeyer flasks. Taking the reaction—



to be reversible, it would follow that, as a result of the insolubility of cadmium sulphide, the reaction



will tend to proceed to the right, thus by the fresh formation of cadmium sulphide increasing the M.S.S.E. at the expense of the thiosulphate present. Under the possible catalytic influence of the cadmium salts in solution the trithionate then reacts with some of the free polysulphide sulphur forming higher polythionates.

With these preliminary remarks we may now proceed to describe method in more detail.

DETAILED ANALYTICAL PROCEDURE.

I. PRELIMINARY TREATMENT.

5-10 c.c. of the diluted polysulphide liquid, containing about 0.5-1.5 per cent. polysulphide sulphur, is pipetted into a solution of 10 c.c. standard cadmium acetate solution plus 20 c.c. of a 10 per cent. ammonium sulphate solution, the Erlenmeyer flask being briskly rotated while letting in the polysulphide solution. The contents are well agitated, and filtered as soon as possible. For duplicate analyses this operation is executed in quadruplicate. The yellow precipitate of cadmium sulphide and free sulphur is well washed with distilled water until thiosulphate-free. The filtrate contains the thiosulphate as well as any other forms of soluble sulphur compounds that may be present such as sulphites, polythionates, etc. The funnel containing the precipitate of $(\text{CdS} + \text{S}, -)$ is then replaced on the original Erlenmeyer, the filter pierced with a glass rod, and all the precipitate washed back into the Erlenmeyer. For this purpose No. 40 Whatman filter paper is specially recommended, since it does not tend to retain some of the precipitate.

II. MONOSULPHIDE SULPHUR EQUIVALENT—M.S.S.E.

Two of the $(\text{CdS} + \text{S}_{-1})$ precipitates are treated with 20 c.c. N/10 iodine solution (excess) and acidified with 10-20 c.c. pure concentrated hydrochloric acid (chlorine-free). The Erlenmeyer is covered, and left to stand for 10-15 minutes. Any lumps remaining are then broken up with a glass rod, and the excess iodine titrated back with N/10 thiosulphate using starch as indicator—

$$\text{Per cent. M.S.S.E.} = \frac{2 \times 10 \times \text{c.c. N/10} \times 32}{2 \times 10 \times 1,000} \text{ gm. S/100 c.c.} \\ (\text{starting with 5 c.c.})$$

III. FREE POLYSULPHIDE SULPHUR—F.P.S.S.

The remaining two $(\text{CdS} + \text{S}_{-1})$ precipitates are treated with 10-15 c.c. of a fresh solution of sodium sulphite* (75 gm. cryst in 500 c.c. water), and 20 c.c. of an ammoniacal ammonium chloride solution [100 gm. $\text{NH}_4\text{Cl} + 100$ c.c. conc. ammonia (Sp. Gr. 0.888) per litre]. The contents of the Erlenmeyer are agitated, and heated for 1 hour on the waterbath. During this time the contents are from time to time agitated with a glass rod, breaking up any lumps visible, and rinsing the sides of the Erlenmeyer with distilled water. 20 c.c. of a strontium chloride solution (100 gm. anhydrous SrCl_2 per liter) are then added, and after 5 minutes the solution is filtered off into a 250 c.c. measuring flask and the precipitate washed with a 1 per cent. ammonia solution. After cooling the filtrate is made up to 250 c.c. A 100 c.c. aliquot† is pipetted out, a few drops methyl red added and acidified with a 10 per cent. tartaric acid solution. The acid solution is then titrated with N/10 iodine using starch as indicator.

$$\text{Per cent. F.P.S.S.} = \frac{2.5 \times 10 \times \text{c.c. N/10} \times 2 \times 32}{10 \times 1,000} \text{ gm. S/100 c.c.} \\ (\text{starting with 5 c.c.})$$

IV. THIOSULPHATE SULPHUR—T.S.S.

The original filtrate [obtained by filtering off $(\text{CdS} + \text{S}_{-1})$ precipitate] is diluted to about 150 c.c., acidified with 20 c.c. 20 per cent. acetic acid, and titrated with N/10 iodine using starch as indicator \rightarrow A c.c. N/10 I. To this titrated solution are added a few drops of phenolphthalein solution, and the solution then made distinctly alkaline with ammonia. About 1 gm. of pure potassium cyanide is then added, the solution agitated, and allowed to stand for half an hour. The solution is then acidified with 25 c.c. sulphuric acid (1:3) and titrated immediately with N/10 iodine, using starch as indicator \rightarrow B c.c. N/10 I.

It will generally be found that 2B approaches A very closely. In case of any appreciable difference between A and 2B in such a way that $A - 2B$ falls out positively, the presence of sulphite is indicated, which is given by—

$$\text{Per cent. sulphite sulphur} = \frac{(A - 2B) \times 2 \times 10 \times 32}{2 \times 10 \times 1,000} \text{ gm. S/100 c.c.} \\ (\text{starting with 5 c.c.})$$

* Owing to the fact that sodium sulphite sometimes contains small quantities of iodine-reducing substances other than sulphite, and also on account of the fact that strontium sulphite is not totally insoluble, it is strongly recommended to control the purity of the reagents used by running blank determinations from time to time. Too great an excess of ammonia must also be avoided since otherwise the reaction becomes sensibly reversible according to the equation:



† If the solution is not perfectly clear it is re-filtered before taking the aliquot.

When, however, $A - 2B$ falls out distinctly negatively, the pre-existence of polythionates is indicated which, taking the tetrathionates as the basis of calculation, are given by—

$$\text{Per cent. Tetrathionate sulphur} = \frac{(2B - A) \times 2 \times 10 \times 2 \times 32}{10 \times 1,000} \text{ gm. S/100 c.c.} \\ \text{(starting with 5 c.c.)}$$

These considerations, however, appear only to apply to special cases. For all practical purposes and for the general control of polysulphide solutions we would tentatively suggest the calculation of thiosulphate sulphur by the formula—

$$\text{Per cent. Thiosulphate sulphur} = \frac{A^1 \times 2 \times 10 \times 2 \times 32}{10 \times 1,000} \text{ gm. S/100 c.c.} \\ \text{(starting with 5 c.c.)}$$

Where A^1 represents the value of A corrected according to the accompanying graph (see Table 2).

V. TOTAL SULPHUR.

5 c.c. Polysulphide solution is pipetted into 20 c.c. 5 per cent. sodium hydroxide solution contained in an Erlenmeyer flask, and the sulphur oxidized to sulphate by adding 10-15 c.c. perhydrol (free of sulphuric acid). The Erlenmeyer is heated on the waterbath for one to two hours, about 5-10 c.c. of bromine water added, and heating continued for about another half an hour. The contents of the Erlenmeyer are then acidified with pure hydrochloric acid, the liberation of bromine acting as indicator, the solution diluted to about 100 c.c., and the bromine expelled by leaving on the waterbath. To the hot, absolutely clear solution 50 c.c. of 10 per cent. barium chloride solution are added, the precipitate allowed to settle on waterbath, and the barium sulphate filtered off through ashless filter paper. The filter with precipitate is ignited in a weighted platinum crucible, the crucible cooled, a few drops of concentrated sulphuric acid added, the acid again expelled by careful heating, and the crucible again ignited and finally weighed.

$$\text{Per cent. total Sulphur} = \text{weight } \text{BaSO}_4 \times .1373 \times 20 \text{ gm. S/100 c.c.}$$

VI. TOTAL CALCIUM.

10 c.c. of the polysulphide solution is pipetted into 20 c.c. 5 per cent. sodium hydroxide solution in an Erlenmeyer, 15 c.c. hydrogen peroxide added, and heated 1 to 2 hours on waterbath. The contents of the Erlenmeyer are then acidified with hydrochloric acid until a clear solution is obtained, 20 c.c. of a saturated solution of ammonium oxalate added, and ammonia added carefully until on brisk agitation the precipitate of calcium oxalate becomes clearly crystalline. After standing another half an hour on the waterbath the precipitate is filtered off by suction through an asbestos pulp filter (asbestos pulp specially treated), washed well with distilled water, and the precipitate washed back into the original Erlenmeyer by breaking up the filter. The oxalic acid is then freed by adding 40 c.c. 10 per cent. sulphuric acid, and the free oxalic acid titrated with $N/10$ potassium permanganate solution.

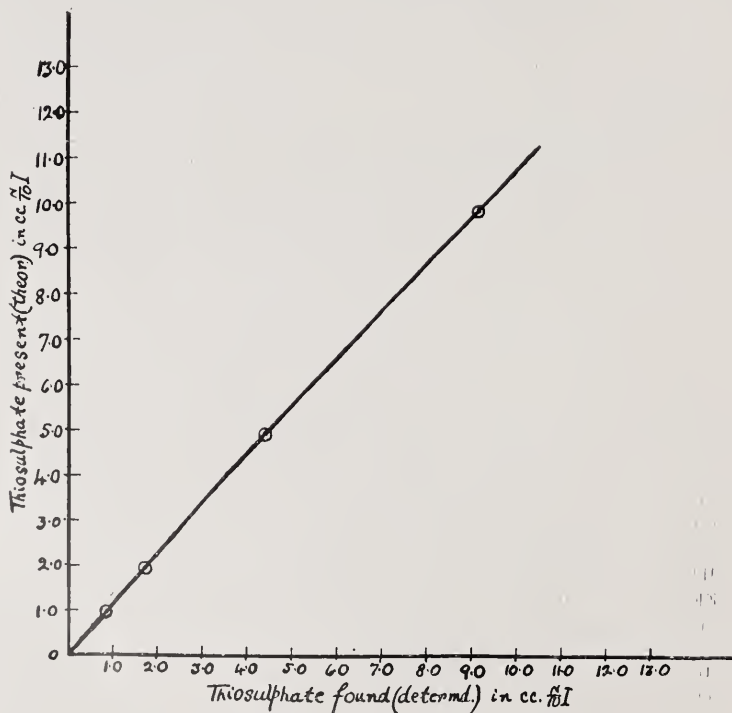
$$\text{Per cent. Calcium} = \frac{\text{c.c. } N/10 \text{ KMnO}_4 \times 40.1 \times 10}{2 \times 10 \times 1,000} \text{ gm. Ca/100 c.c.}$$

RE-AGENTS.

Cadmium Acetate Solution.—112 gm. CdCO_3 (Merck's extra pure) are weighed out into a 1000 c.c. Erlenmeyer flask, and wetted with 200 c.c. distilled water. To this are added 135 c.c. of glacial acetic acid in small portions, shaking well after the addition of every portion. The Erlenmeyer is then left to stand one to two days at room temperature. After this treatment the Erlenmeyer can be heated on the waterbath (one to two days) and then heated on a wire gauze till all the CdCO_3 is dissolved without the danger of frothing up. The solution is filtered and diluted to 1,500 c.c.

TREATMENT OF ASBESTOS PULP.

A quantity of Merck's asbestos pulp for Gooch crucibles is treated in an Erlenmeyer with an acid solution of potassium permanganate, the Erlenmeyer being heated a few hours on the waterbath. The permanganate is then filtered off under suction through a glass funnel, and the asbestos washed with water until all the permanganate has been removed. The mass, now brown in colour, is then washed with a solution of sodium sulphite in dilute sulphuric acid until snow white, the excess sulphite removed by washing with dilute sulphuric acid until the filtrate no longer discolours permanganate, and then washed with a dilute solution of hydrogen peroxide. Finally the asbestos is well washed with distilled water, and transferred with distilled water into the storage bottle.



SUMMARY.

A review of the literature relating to the analysis of sulphides, polysulphides and allied substances is given. Various analytical methods have been tested while others are still under investigation. For the analysis of field and laboratory polysulphide solutions a new volumetric cadmium acetate method has been evolved the mono-sulphide equivalent being precipitated as cadmium sulphide and titrated iodometrically while the free polysulphide sulphur is titrated as thiosulphate after conversion with sulphite. The thio-sulphate is titrated in the filtrate, while the total sulphur is determined gravimetrically. Some of the reactions involved have been studied experimentally.

LITERATURE.

- A.O.A.C. (1916). *Journal of A.O.A.C.* II, May 15, 1916, p. 18.
 A.O.A.C. (1920). *Journal of A.O.A.C.* III, Febr. 15, 1920, p. 353.
 ATKIN (1922). *J. Soc. Leather Trades' Chem.* No. 6, p. 427. *Chem. Abstr.* No. 17, p. 116 (1923).
 BODNAR (1914-15). *Chem. Ztg.* No. 39, p. 715 (1915). *Z. anal. Chem.* No. 53, p. 37 (1914).
 CALCAGNI (1921). *Gazz. Chim. ital.* 50, Vol. 2, p. 331. *Centralblatt* 1921, Vol. 1, p. 931.
 CHANCEL and DIACON (1863). *J. prakt. Chem.* No. 90, p. 55.
 CHAPIN (1916A). *Ind. Eng. Chem.* No. 8, p. 151.
 CHAPIN (1916B). *Ind. Eng. Chem.* No. 8, pp. 151 and 339.
 FOCK and KLÜSS (1890). *B. 23*, p. 534.
 GOODWIN and MARTIN (1925). *J. Agr. Science*, No. 15, p. 96.
 HARRIS (1911). *Michigan Agr. College Techn. Bull.*, No. 6.
 JONES (1923). *J. Agr. Research*, No. 25, p. 323.
 KURTENACKER and BITTNER (1924). *Z. anorg. allg. Chem.*, No. 141, p. 297.
 KURTENACKER and BITTNER (1925). *Z. anorg. allg. Chem.*, No. 142, p. 115.
 KURTENACKER and FRITSCH (1921). *Z. anorg. allg. Chem.*, No. 117, p. 262.
 KURTENACKER and GOLDBACH (1927). *Z. anorg. allg. Chem.*, No. 166, p. 177. See also Ishikawa (1927) *Z. physikal. Chem.*, No. 130, p. 73.
 KURTENACKER and KAUFMANN (1925). *Z. anorg. allg. Chem.*, No. 148, p. 256.
 MÜLLER (1915). *Bull. Soc. Chim. de France*, Vol. 4, No. 19, p. 8. *Centralblatt* 1916, Vol. 1, p. 598.
 SANDER (1915). *Chem. Ztg.*, No. 39, p. 945. See also *Z., angew. Chem.*, No. 28, p. 9 (1914).
 SCHULEK (1925). *Z. anal. Chem.*, No. 65, p. 352.
 SHIMO and MAKITA (1929). *J. Soc. Chem. Ind. Japan.*, No. 32, p. 123. *Chem. Abstr.*, No. 23, p. 4907 (1929).
 SMITH and TAKAMATSU (1882). *Trans. Chem. Soc.*, No. 11, p. 162.
 SZEBERENYI (1929). *Z. anal. Chem.*, No. 78, p. 36.
 WATSON and RAJAGOPALAN (1922). *Proc. Asiatic Soc. Bengal*, Vol. 18, No. 6, p. 66. *Chem. Abstr.*, No. 18, p. 1255 (1924).
 WÖBER (1917). *Chem. Ztg.*, No. 41, p. 569.
 WOLLAK (1929). *Z. anal. Chem.*, No. 77, p. 401.

Researches into Dips and Dipping.

A.—Lime-Sulphur Dips.

Paper III.—A Preliminary Study of a Colorimetric Method as a rapid means of control of Polysulphide Solutions.*

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IN spite of the general use of lime-sulphur (calcium polysulphide) solutions as an effective dip against scab, there exists as yet no simple and effective method for the control of lime-sulphur tank-washes in the field. The analytical methods are all relatively complicated, and incapable of easy application by farmers and stock inspectors under field conditions. As far as the author is aware, the only field-method so far suggested is that of Chapin (1915) involving the principle of titration with iodine with sodium nitroprusside as indicator. For reasons given in the preceding paper (II), this idiometrical titration is theoretically correct when using an indicator responsive to the sulphide ion. Moreover, in the hands of an experienced chemist quite satisfactory results may be obtained when using uncontaminated lime-sulphur solutions, the nitroprusside colour changing from purple-violet to a dirty green in the course of the titration. Just before reaching the end-point, however, the colour changes to a distinct blue, which disappears relatively sharply on reaching the end-point. On the other hand, solutions contaminated during the process of dipping cause difficulty, the end-point becoming largely a question of guess-work. It would be little less than courting confusion to place such a method in the hands of those uninitiated in the manipulations of chemical analyses.

Moreover, such a method determines merely the monosulphide equivalent, and gives no indication whatsoever of the amount of polysulphide actually present. It has been customary to calculate the total polysulphide from the monosulphide equivalent so determined by introducing the empirical factor 4.6, assuming the polysulphides of calcium in solution to approach the formula CaS_x where x is taken to exhibit the mean value of 4.6.

On the factors which determine and modify the value of x we shall report in a later paper. In passing, however, we should like to point out that we have found dips where, as a result of dipping, the value of x had fallen to something in the neighbourhood of 2.0.

* This work has been carried out with the aid of a grant from the Empire Marketing Board.

Taking an hypothetical example, it would mean that a dip, registered by the above method as containing 0.9 per cent. polysulphide sulphur, would in reality contain only about 0.4 per cent. disulphide sulphur. It would therefore appear that, as far as our present knowledge goes, the purely chemical methods of analyses capable of rendering the required information all become so involved and complicated that they become impracticable under field conditions.

From the fact, however, that the higher polysulphides give solutions from orange to orange-brown to almost black in colour, while the disulphide gives a yellow coloration and the monosulphide and other inorganic sulphur compounds are usually colourless, it was thought desirable to determine colorimetrically the relationship between colour intensity and concentration of polysulphide solutions. Little is known concerning the nature of the chromophore of such solutions. It would, however, appear that under certain definite conditions relating to composition such solutions conform to Beers Law. Using a Leitz colorimeter, solutions of different polysulphide concentration were compared colorimetrically. These solutions were prepared by diluting down a proprietary lime-sulphur concentrate containing 35.5 per cent. polysulphide sulphur with a value of x equal to 4.5. The dilutions, represented as K/10, K/20, K/40, K/50 and K/100 hence represent concentrations of 3.55, 1.77, 0.89, 0.71 and 0.35 per cent. polysulphide sulphur respectively. In order to avoid atmospheric oxidation, the solution in the colorimeter cup, after having immersed the glass column, was covered with a thin layer of 70-80° benzine. The colorimetric readings so obtained have been tabulated in Table 1.

TABLE 1.

L/R	L.	R.	Le/Rc
$\frac{K}{10} / \frac{K}{20}$	$\begin{cases} 5.0 \\ 2.0 \end{cases}$	$\begin{cases} 10.4 \\ 3.8 \end{cases}$	$\begin{cases} 1 : 2.08 \\ 1 : 1.9 \end{cases}$
$\frac{K}{10} / \frac{K}{40}$	$\begin{cases} 5.0 \\ 2.0 \end{cases}$	$\begin{cases} 20.4 \\ 8.2 \end{cases}$	$\begin{cases} 1 : 4.08 \\ 1 : 4.1 \end{cases}$
$\frac{K}{10} / \frac{K}{50}$	$\begin{cases} 4.0 \\ 2.0 \end{cases}$	$\begin{cases} 19.5 \\ 10.0 \end{cases}$	$\begin{cases} 1 : 4.9 \\ 1 : 5.0 \end{cases}$
$\frac{K}{20} / \frac{K}{40}$	$\begin{cases} 5.0 \\ 2.0 \end{cases}$	$\begin{cases} 10.4 \\ 4.0 \end{cases}$	$\begin{cases} 1 : 2.08 \\ 1 : 2.0 \end{cases}$
$\frac{K}{20} / \frac{K}{100}$	$\begin{cases} 5.0 \\ 2.0 \end{cases}$	$\begin{cases} 24.0 \\ 9.5 \end{cases}$	$\begin{cases} 1 : 4.80 \\ 1 : 4.75 \end{cases}$
$\frac{K}{40} / \frac{K}{100}$	$\begin{cases} 5.0 \\ 2.0 \\ 10.0 \end{cases}$	$\begin{cases} 12.0 \\ 5.0 \\ 25.0 \end{cases}$	$\begin{cases} 1 : 2.40 \\ 1 : 2.50 \\ 1 : 2.50 \end{cases}$

From the above table it will be seen that the colour intensity is proportional to the concentration. In order to obtain the necessary

sensitivity due to strong absorption, it was found necessary to work with thin layers in lieu of low concentrations. It is evident, however, that in view of the great instability of polysulphide solutions, such a colorimetric method would be of little use unless some colorimetric standard for comparison of sufficient stability could be found.

For this purpose a solution of pure chromic acid in water was found most suitable, the solution in all practical dilutions giving exactly the same colour tint in the colorimeter as the corresponding polysulphide solutions. A stock solution—SS. of Merck's chromic acid (extra pure) was prepared containing 12.984 gm. CrO_3 per 100 c.c. solution, and representing about a concentration (computed from SS/10 solution) of 40 per cent. polysulphide sulphur. In order to determine this polysulphide equivalent (colorimetric) of the standard chromic acid solution, the stock solution SS. was diluted to one in ten, and the different concentrations of polysulphide measured against this SS/10 solution of chromic acid. The results obtained have been tabulated in Table 2.

TABLE 2.

L/R SS 10/ Dip	L.	R.	SS /K 10/ 10
SS /K 10/ 100	2.0	23.0	1.15
SS /K 10/ 50	{ 5.0 2.0	{ 27.5 12.0	{ 1.10 1.20
SS /K 10/ 40	{ 5.0 2.0	{ 22.0 10.5	{ 1.11 1.10
SS /K 10/ 20	{ 5.0 2.0	{ 10.5 4.6	{ 1.05 1.15
SS /K 10/ 10	{ 5.0 2.0	{ 6.0 2.5	{ 1.20 1.25

From the observation that the colour intensity of polysulphide solutions is proportional to the concentration, the average relationship of SS/10 to K/10 was determined and found to be 1.15. In other words:—

$$\frac{\text{SS}}{10} \div \frac{\text{K}}{10} = \frac{1.15}{1.00}$$

$$\text{i.e. } \frac{\text{SS}}{10} = 1.15 \times \frac{\text{K}}{10}$$

Hence $\frac{\text{SS}}{10}$ represents $\frac{35.5 \times 1.15}{10}$ or (3.55×1.15) gm. polysulphide sulphur per 100 c.c.

In view of the fact, however, that the concentrations usually employed in dipping practice rarely exceed 1.5 per cent., and usually fall in the vicinity of 1.0 per cent. and lower, the chromic acid standard used for the actual determination of dipping fluids was diluted to one in forty. Comparing this $\frac{\text{SS}}{10}$ solution colorimetrically with the $\frac{\text{SS}}{10}$ solution under conditions identical to those preceding it was found that they exhibited the colorimetric ratio 1/4.3* instead of the theoretical ratio 1/4.0. The $\frac{\text{SS}}{40}$ chromic standard therefore represents $\frac{1.15 \times 3.55}{4.3}$ or 0.95 gm. polysulphide sulphur per 100 c.c. solution. In reading dip fluids against this $\frac{\text{SS}}{40}$ standard the percentage polysulphide sulphur is given by:—

$$\frac{L \times \text{SS}/40}{R} = \frac{0.95 L}{R}$$

In Table 3 are tabulated the results obtained with six dips specially prepared in the laboratory from different grades of commercial lime, the actual amounts of polysulphide sulphur present having been determined by the cadmium acetate method described in Paper A. II. of this series.

TABLE 3.

Dip Solution.	L.	R. Average Reading.	% Poly. S. Found.	% Poly. S. Present.	x (in CaS_x).	
1.....	{ 10.0 5.0	{ 34 20	{ 0.28 0.24	0.26%	0.25%	4.83
2.....	{ 10.0 5.0	{ 27 15	{ 0.35 0.32	0.34%	0.34%	4.87
3.....	{ 10.0 5.0	{ 20 11.5	{ 0.47 0.41	0.44%	0.43%	4.98
4.....	{ 10.0 5.0	{ 16 8.5	{ 0.59 0.56	0.57%	0.56%	5.12
5.....	{ 10.0 5.0	{ 14.5 7.5	{ 0.65 0.63	0.64%	0.63%	4.80
6.....	{ 10.0 5.0	{ 14.0 8.0	{ 0.68 0.60	0.64%	0.68%	5.37

* This diversion from Beer's Law is most probably due to the fact that, as suggested by Hantzsch [*Z. physikal. Chem.*, 63, 367 (1908) and 72, 362 (1910)], chromic acid resp. chromic acid anhydride exists in the solid phase in a highly polymerized form. On entering into solution this molecular association is apparently partially retained in conc. solutions. On dilution molecular dissociation proceeds rapidly until in dilute solutions only dichromic acid exists in solution.

From the table it is clear that, as a rapid means of control, the colorimetric method gives highly satisfactory results. We note, however, that in all these solutions the value of x in CaS_x lies above 4.6. It therefore appeared of practical interest to determine to what extent the colorimetric readings are influenced by the value of x , that is, by the nature of the polysulphides present.

For this purpose a set of solutions of decreasing x was prepared by passing pure carbon dioxide through a standard polysulphide solution. The results obtained by the colorimetric analysis as compared with those given by the cadmium acetate method have been tabulated in Table 4.

TABLE 4.

Polysulphide Solution.	L.	R. Average Reading.	% Poly. S. Found.	% Poly. S. Present.	% Deviation.	x (in CaS_x H_2S_x).
I.....		Standardization	1.42%	1.42%	0%	4.5
II.....	{ 10.0	{ 9.2	{ 1.03	1.22%	-18%	4.1
	{ 5.0	{ 5.0	{ 0.95			
III.....	{ 10.0	{ 16.0	{ 0.59	0.77%	-27.3%	3.3
	{ 5.0	{ 9.0	{ 0.53			
IV.....	{ 10.0	{ 22.0	{ 0.43	0.60%	-33.3%	2.8
	{ 5.0	{ 12.5	{ 0.38			
V.....	{ 10.0	{ 34	{ 0.28	0.41%	-36.6%	2.7
	{ 5.0	{ 19	{ 0.25			

It is therefore clear that, with solutions in which the value of x falls appreciably below a certain critical value—apparently 4.5-4.6, the values obtained by the colorimetric method fall out too low, the percentage deviation from the true value increasing as x decreases. In connection with the composition and structure of polysulphides in solution this observation appears to possess special theoretical importance, though at this stage we have not collected sufficient data to warrant any theoretical discussions. The same caution must be exercised in using the existing colorimetric relationship between the chromic acid chromophore and the polysulphide chromophore as a means of arriving at any conclusions regarding the structure of polysulphides in solution. Since it has been shown (Levi and Baroni, 1929) that the alkyl polysulphides exist in different isomeric forms, the possibility that the inorganic polysulphides may also be subject to isomerism becomes extremely probable. This isomerism apparently also holds for aryl-substituted polythionic acids as has been shown by Christiansen (1928). Since, in the opinion of the writer, the polythionic acids must be considered as the primary autoxidation products of polysulphides in aqueous solution, and since the conditions favourable for the existence of particular isomers are as yet not known, the interpretation of results must await further investigation.

In accordance with these considerations the colorimetric determination was applied to a number of home-made lime-sulphur dips obtained from various sources as described in our introductory paper. These results have been tabulated in Table 5.

COLORIMETRIC ANALYSIS OF LIME-SULPHUR DIPS.

TABLE 5.

Dip.	L.	R. Average Reading.	% Poly. S. Found.	% Poly. S. Present.	x (in CaS ₂).
1.....	—	—	—	—	—
2.....	{ 10.0 5.0	{ 15.5 7.8	{ 0.61 0.61	{ 0.61% 0.68%	4.8
3.....	{ 10.0 5.0	{ 11.5 5.8	{ 0.80 0.82	{ 0.81% 0.95%	4.6
6.....	{ 10.0 5.0 2.0	{ 17.5 9.3 3.6	{ 0.54 0.51 0.53	{ 0.53% 0.80%	4.4
A. 7.....	{ 10.0 2.0	{ 12.0 2.5	{ 0.76 0.79	{ 0.78% 0.96%	4.5
8.....	{ 10.0 5.0 5.0	{ 10.3 5.5 29.0	{ 0.92 0.86 0.16	{ 0.89% 1.20%	4.9
9.....	{ 5.0 2.0	{ 29.0 11.0	{ 0.16 0.17	{ 0.17% 0.30%	4.2
1.....	{ 10.0 5.0 10.0	{ 9.5 5.0 8.5	{ 1.00 0.95 1.12	{ 0.97% 0.97%	4.8
2.....	{ 10.0 5.0	{ 8.5 4.4	{ 1.12 1.09	{ 1.11% 1.16%	4.7
B. 3.....	{ 10.0 5.0	{ 8.3 4.5	{ 1.14 1.06	{ 1.10% 1.23%	3.6
6.....	{ 10.0 2.0	{ 12.0 2.4	{ 0.79 0.79	{ 0.79% 1.67%	3.6
7.....	{ 10.0 5.0	{ 16.0 9.5	{ 0.59 0.50	{ 0.55% 1.27%*	4.3
8.....	{ 10.0 5.0	{ 13.0 6.5	{ 0.73 0.73	{ 0.73% 1.17%	4.8
6.....	{ 20.0 10.0	{ 4.2 2.8	{ 4.50 3.40	{ 3.95% 1.39%	4.6
7.....	{ 10.0 5.0	{ 4.0 2.5	{ 2.37 1.90	{ 2.13% 2.75%*	4.4
C. 10.....	{ 20.0 10.0	{ 5.2 3.2	{ 3.65 2.97	{ 3.31% 2.75%	4.7
11.....	{ 10.0 5.0	{ 10.0 6.1	{ 0.95 0.78	{ 0.87% 0.80%	4.6
12.....	{ 10.0 5.0	{ 11.0 7.0	{ 0.86 0.68	{ 0.77% 0.53%	4.6

* Dip strengthened by the addition of fresh concentrate during the course of dipping.

In this table Group A represents home-made concentrates diluted down in the laboratory with distilled water. Group B represents the diluted solutions of A as received (diluted down to dipping strength in the field), and Group C represents dip fluids in which dipping had taken place. In selecting the samples classed under Group C great care was taken to select only those giving absolutely clear solutions on sedimentation. Dips Nos. 1, 2, 3 and 6 were prepared from the same lime as the corresponding dips (prepared in the laboratory) in Table 3. It will be observed that both Groups A and B exhibit a strong tendency to give colorimetric readings appreciably below the actual amount of polysulphide sulphur present. In contrast to this the dips falling under Group C, that is dips subjected to actual dipping, show a distinct tendency to give colorimetric values appreciably too high. By investigating, both by physical

and chemical methods, the constitution of polysulphides in solution and their oxidation mechanism it is hoped to obtain a better insight into these phenomena.

SUMMARY.

A solution of pure chromic acid in water was found to be well suited as a standard of comparison for the colorimetric determination of calcium polysulphides in solution. With certain polysulphide solutions under adequately controlled conditions the colorimetric comparison method yields excellent results, whereas other polysulphide solutions give both low and high values depending on various factors as yet not fully elucidated.

In conclusion I should like to express my obligation to my assistant, Mr. H. A. Hambrock, B.Sc.(Agric.), for determining by means of the cadmium acetate method, the polysulphide sulphur content of the dips tabulated in Table 5.

LITERATURE.

- CHAPIN (1915). A field test for Lime-sulphur Dipping Baths. *Bull. U.S. Dep. Agr.*, No. 163.
- LEVI and BARONI (1929). *Atti accad. Lincei*, Vol. 6, No. 9, pp. 772 and 903. *Chem. Abstr.*, Vol. 23, pp. 4927 and 5158 (1929).
- CHRISTIANSEN (1928). *Z. Elektrochem.*, No. 34, p. 638.

Section X.

Miscellaneous.

- J. QUINLAN AND G. S. MARÉ. The Normal Temperature of Merino Sheep during January in the Karroo, and how it is Influenced by Exercise.
- H. H. CURSON ... Notes on the Flora of Ngamiland and Chobe. Part I. Outline of the Floral Regions.
- N. T. v. D. LINDE A Peculiar Case of Traumatism affecting the Metatarsal Bones
- G. C. VAN DRIMMELEN AND A. R. THIEL. Anatomical Studies, No. 28. Hypospadias in a Merino Ram.
- H. H. CURSON ... Anatomical Studies, N. 29. A further Note on Free-Martinism.
- H. H. CURSON ... Anatomical Studies, No. 30. On Two Cases of Atresia Ani.
- H. H. CURSON ... Anatomical Studies No. 31. On Two Cases of Acardiacus.
- W. D. MALHERBE Anatomical Studies No. 32. Atresia Ani with Rectum opening into Vagina in a Kitten.
- W. J. WHEELER Anatomical Studies No. 33. Micrognathia in a Lamb.
- G. S. MARÉ ... Anatomical Studies No. 34. Faulty Jaws in Sheep.
- I. P. MARAIS ... Anatomical Studies No. 35. On the Origin of an Abdominal Cyst found in a Domestic Hen.
- H. H. CURSON ... Anatomical Studies No. 36. On Two Anomalies of the Cervix Uteri in a Merino Sheep.
- R. BIGALKE ... Anatomical Studies No. 37. On a Hybrid Duiker.

The Normal Temperature of Merino Sheep during January in the Karroo, and how it is Influenced by Exercise.

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THE object of this experiment was to ascertain the daily variation in body-temperature of normal Merino ewes maintained under Karroo conditions.

The ten animals used in the experiment were selected from the flock maintained at the Grootfontein School of Agriculture. They ranged in ages from three to four years, carried a four months' fleece, and were in good store condition.

While the experiment was in progress the sheep were confined to an enclosure, measuring 36 feet by 28 feet, in which a small, well ventilated wood and iron shed afforded shade. The ration fed consisted of lucerne hay, mealies, and whole oats. A plentiful supply of fresh, clean water was available within the enclosure.

Clinical thermometers, which had previously been standardised, were used to register the rectal temperature. Two thermometers were inserted at a time and the average reading was recorded after the necessary corrections had been made. The difference in temperature as registered by two half-minute thermometers inserted simultaneously and kept in the rectum for 2 minutes, seldom exceeded 0.2° F.

For the first five days the temperatures were taken at 6 a.m. and 2 p.m. and for the next seven days an additional reading was recorded at 7.30 p.m. daily. The air temperature was noted each time the animals were tested. For this purpose a thermometer was suspended from one of the beams in the small shed.

In Table 1 the mean temperature of each animal is recorded. The table also shows the highest and lowest temperatures registered for each individual during the 12 days that the experiment lasted.

TABLE 1.

Ewe No.	Temperatures Fahrenheit Scale.				
	At 6 a.m.	At 2 p.m.	At 7.30 p.m.	Highest.	Lowest.
MX5.....	101.85	102.53	103.01	104.0	101.0
061.....	101.47	102.91	102.74	103.6	100.8
080.....	101.65	102.38	102.43	103.0	101.0
096.....	101.85	102.74	103.03	103.4	101.5
173.....	101.09	102.29	102.83	104.0	100.4
174.....	101.97	102.77	102.73	103.9	101.4
187.....	102.95	103.28	103.62	105.4	101.2
193.....	101.54	102.55	102.56	104.2	101.0
0 209.....	101.38	102.90	103.39	104.6	100.6
0 213.....	101.91	102.87	103.14	103.8	101.1
MEAN.....	101.77	102.72	102.95	—	—

The figures in the above table show:—

- (1) That in every case the body-temperature increased from 6 a.m. to 2 p.m., the average increase being 0.95° F.
- (2) A progressive increase of temperature from 6 a.m. to 7.30 p.m. amounting to 1.18° F.
- (3) An appreciable individual variation in normal temperature; ewe No. 187 registered the highest mean temperature.
- (4) A variation in temperature for the ten ewes from 100.4° to 105.4° F.

In Table 2 the average daily temperatures of the ten ewes are recorded. The air temperatures are included in the table together with the official maximum and minimum readings.

TABLE 2.

Temperatures Fahrenheit Scale.								
Date January.	At 6 a.m.		At 2 p.m.		At 7.30 p.m.		Air.	
	Ewes.	Air.	Ewes.	Air.	Ewes.	Air.	Max.	Min.
13.....	101.78	57	102.58	84	—	—	86	50
14.....	101.76	62	102.72	93	—	—	93	53
15.....	101.95	66	103.11	88	—	—	86	60
16.....	101.89	63	102.46	77	—	—	75	56
17.....	101.66	53	102.46	87	—	—	84	41
18.....	101.59	56	102.60	87	102.98	72	85	50
19.....	101.76	55	102.67	84	102.68	71	85	48
20.....	101.40	60	102.28	85	102.71	76	84	51
21.....	101.62	62	102.84	91	103.09	77	92	54
22.....	101.84	65	102.90	95	102.53	65	89	62
23.....	101.60	58	102.76	96	102.94	84	92	52
24.....	102.35	67	103.20	100	103.57	86	94	60
AVERAGE...	101.77	60.3	102.72	89.0	102.95	76.0	87.0	53.0

As in the case of Table 1 the above figures also show a progressive rise in body-temperature from 6 a.m. to 7.30 p.m. The body-temperature does not, however, vary directly according to air-temperature. The latter was lowest at 6 a.m., highest at 2 p.m., and intermediate at 7.30 p.m.; while the body-temperature was highest at 7.30 p.m.

Furthermore the body-temperature did not respond to the air-temperature in respect of hot or cold days and nights.

The ewes were teased daily at 6.30 a.m. for oestrus. Only ewe O 209 showed oestrus during the 12 days. Her temperature was apparently not disturbed as is indicated in Table 3, which gives the readings two days before, two days during, and two days after oestrus.

The following table shows the temperature of ewe O 209 two days prior to oestrus, two days during oestrus, and two days subsequent to oestrus.

TABLE 3.

January.	6 a.m.	2 p.m.	7.30 p.m.
18th.....	101.35	101.85	102.75
19th.....	100.95	102.80	102.75
20th.....	100.70	102.35	103.70
21st.....	101.45	103.60	103.85
22nd.....	101.65	103.90	102.80
23rd.....	101.25	103.00	103.35

Conclusion.—It must be concluded that, provided a normal Merino ewe is in a restful state, her temperature will vary, in warm summer weather, during the course of the day, being highest at night and lowest in the early morning. Normal fluctuations in air-temperature will not affect the body-temperature.

On the termination of the above experiment a test was carried out with ten ewes to ascertain to what extent body-temperature could be influenced by excitement and exercise. The test was conducted from 2-2.45 p.m. in bright sunshine. The shade-temperature was 81° F.

In Table 4 the results of the test are recorded:—

TABLE 4.

Ewe No.	Treatment.	Temp. before treatment.	Temp. after treatment.	Increase.
061	Frightened, cornered, and caught in small enclosure.	102.6	103.8	1.2
080	Frightened, cornered, and caught in small enclosure.	102.3	103.0	0.7
187	Driven 200 yards*.....	103.2	103.5	0.3
0213	Driven 200 yards.....	102.4	103.0	0.6
MX5	Driven 400 yards.....	102.3	103.1	0.8
0209	Driven 400 yards.....	103.2	103.6	0.4
173	Driven 800 yards.....	103.2	104.1	0.9
174	Driven 800 yards.....	102.8	104.1	1.3
193	Driven 1,600 yards.....	102.4	104.8	2.4
096	Driven 1,600 yards.....	102.4	105.2	2.8

* The ewes were driven at a walking pace of 3.4 miles per hour, two at a time.

The results show conclusively that a progressive rise in temperature took place according to the distance driven. Sudden exertion and excitement also caused a rise in temperature.

Notes on the Flora of Ngamiland and Chobe.

Part I: Outline of the Floral Regions (1).

By Dr. H. H. CURSON, F.R.C.V.S., Veterinary Research Officer,
Onderstepoort.

(Seconded to Bechuanaland Protectorate Government for Glossina
Investigations from November 1930–February 1931.)

Introduction.

Environmental factors.

Vegetation areas.

General description of each type.

Poisonous plants.

Summary.

Appreciation.

References.

With one map and 15 figures.

INTRODUCTION.

THE object of this paper is to give some idea of the plant geography of the Ngamiland and Chobe magisterial districts, especially to those interested in Animal Husbandry. In a consideration not only of the Glossina problem (Curson 1932), but also of other problems in Animal Husbandry, some knowledge of the environment is essential, and hitherto nothing has been attempted for the area in question.

It is recognized that plant geographies, unless described in detail, may be misleading, for in most territories there is so much variation, particularly of soil conditions, that a map is, after all, very approximate. For example, the northern portion of the Pretoria magisterial district may be classed as woodland, but within a short distance of approximately sixty miles along the Pretoria-Warmbaths road, no less than half a dozen types varying from southern Kalahari flora to a broad-leaf deciduous type may be encountered. In the northern part of Bechuanaland Protectorate there is, however, more uniformity in soil conditions and climate, thus making an attempt to describe the general floral types entirely justifiable.

(¹) Part II, by Mr. A. O. D. Mogg, Division of Plant Industry, Pretoria, will deal with a list of plants collected during a tour around the Okavango Delta with a discussion thereon.

ENVIRONMENTAL FACTORS.*(a) TOPOGRAPHY.*

Ngamiland and Chobe, with an area of over 25,000 square miles, form the north-western portion of the northern half of the Bechuanaland Protectorate. The territory, an extensive sandy plain belonging to the Kalahari System and having an altitude of slightly over 3,000 feet, is remarkable on account of the Okovango Delta. The Okovango River, rising in the Angola Highlands, flows, not to the Atlantic Ocean, but south-east, and after a course of probably 1,000 miles enters Ngamiland where it divides into numerous branches, constituting the Okovango Delta. Here it expends most of its flow and whatever remains after evaporation and seepage is finally collected by the Mogogelo and Thamalakane Rivers which together form the base of the Delta. During exceptional floods not only may the Mogogelo and Thamalakane Rivers communicate north and south with the Mababe Depression and Lake Ngami respectively, but sufficient water may be available for flow to the Makarikari Depression.

Du Toit (1927) assumes, in regard to the delta-formation of the Okovango, that formerly the River "discharged into the Limpopo via the Macloutsie River probably." Then occurred several "important crustal movements" among which was an uplifting from Marandellas in Southern Rhodesia to immediately "south of the Makarikari", giving rise to the "straight watershed of Southern Rhodesia" and the Makarikari Depression "with the consequent damming back of the water (of the Okovango) to form a great lake in which the entire flow of the Okovango River was consumed by evaporation". Following this was a subsidence of the earth's crust "in the Ngamiland region . . . athwart the Okovango-Linyanti (Chobe)-Zambesi river systems" which not only disorganized them but which deprived "the Botletle River (Okovango) of its normal supplies", whereupon the Makarikari was replaced by this great depression, called by Schwarz "Greater (Lake) Ngami". Later, reclamation took place with the result that the Zambesi and Linyanti (Chobe) Rivers were enabled to wend their way across the silted plain to meet, and finally to hurl their united waters over the mighty Victoria Falls.

Apart from the Chobe River which forms part of the northern boundary and, of course, the Okovango River, there is no other permanent water, except a few isolated pools, e.g. Tsotsoroga Pan. Almost the entire native population, numbering approximately 25,000, lives near the alluvial banks of the above rivers. Away from these the country is a bush-covered waste occupied by a few wandering Bushmen.

It is thus clear that the Okovango and Chobe Rivers provide the life-blood of the territory under consideration.

(b) CLIMATE.

Rainfall.—Over 95 per cent. of the rain falls between the months of September and March, especially during December and January. From May to August it seldom rains. The average annual rainfall ranges from 23.15 inches (1915-1923) at Andara and 29.95 inches at Kasane (1928-1930), both in the north, through 14.12 inches at Tsau

(1911-1916) and 14.75 inches at Maun (1928-1930), both centrally situated, to 16.14 inches at Ghansi (1928-1930) in the south. Owing to the sandy nature of the country all rain is absorbed, except, of course, from pools where evaporation is marked.

Coinciding with the dry winter are the winter floods due to the heavy summer rainfall (in the Angola Highlands), but which owing to the flatness of the country take many months to reach the Thamalakane River.

Temperature.—The mean maximum temperature ranges from an average (1928-1930) of 78.3° in July to 97.1° in October at Kasane. The corresponding mean minimum records are 44.3° and 66° . At Maun in 1930 the mean maximum was 76° in July and 96.3° in October and the parallel minimum figures were 39° and 61.9° . At Ghansi the mean maximum records for the same months from 1928-1930 were 74.1° and 92° respectively and the mean minimum temperatures 37.8° and 60.3° .

The highest absolute maximum recorded during the above period was 110° at Maun in November, 1930, and the lowest absolute minimum was 24° registered at Maun in August, 1930, and at Ghansi in June, 1928.

Relative Humidity.—A few figures are given in the Report of the Kalahari Reconnaissance of 1925 (p. 64—Du Toit 1926), but entirely reliable records over a long period are not available. From a practical aspect, the most noteworthy feature of humidity is the beneficial effect of perceptible moisture on the surrounding vegetation, e.g. the spray from the Victoria Falls. Along the swamps, where one might expect the flora of the surrounding Open Tree Country to benefit from what one might term imperceptible moisture, the desert nature of the vegetation is striking.

Winds.—In November, 1930, the heavy winds ushering the rainy season were from a northerly direction and brought much sand. On November 30th occurred a severe sandstorm from the south-east. According to the Report of the Kalahari Reconnaissance of 1925 “the prevailing winds range from E. to E.S.E. and S.S.E.”.

VEGETATION AREAS.

The southern part of Ngamiland is situated in Pole Evans' (1931) Kalahari Thorn Country. In his Vegetation Map of South Africa, Pole Evans shows for the Southern Kalahari two main floral types, (1) the Kalahari Thorn Country (Desert), occupying the greater portion of Bechuanaland Protectorate and forming an enclave or indentation between the south-eastern and south-western extensions of the Central African Savanna to which he refers; and (2) Savanna, Open Tree Country or Bushveld (Grassland) along the eastern portion of Bechuanaland Protectorate, this indeed being the south-eastern arm extending southwards from Central Africa. The south-western extension is to be found in South-West Africa Protectorate, stretching southwards, according to the above authority, as far as Windhoek.

In a map of the grasslands of South Africa, Bews (1918) includes the area under review in his Sandveld of the Kalahari and Central Region, and adds, "The treeless Grass Veld of this region in many places develops into Tree Veld," and then again, "In the northern Kalahari, the Tree Veld is still more tropical in its nature, with *Copaifera mopane*, *Adansonia digitata*, *Peltophorum africanum*, *Strychnos* sp., etc." Actually this type of vegetation, the Savanna of Pole Evans, is to be found in the Middle Kalahari, the term Northern Kalahari in this paper being used for that part of the Great Kalahari Region north of the Chobe River to the Zambesi-Congo Watershed. The Middle Kalahari is, therefore, the country situated between the Chobe River, and approximately the latitude passing through the southern end of Lake Ngami, or in other words, that part of the Kalahari including and adjacent to the remarkable Okovango Delta.

As stated above, Kalahari Thorn Country occurs in the southern portion of Ngamiland. It extends roughly as far north as the Botletle River and from here in a north-westerly direction as far as the Tshodilo Hills. The indentation here described is deep and narrow and corresponds to the area about the south-western portion of the Okovango Delta where desiccation has been so marked within recent times. The extent is difficult to chart for the reason that it has not been studied in detail personally. Information, however, is available from Passarge's description of the Middle Kalahari and Stigand's (1923) map.

The line of division between the Kalahari Thorn Country and Savanna is distinct, especially in the vicinity of the Okovango Delta, but it is understood that east, in the Bamangwato Native Reserve, and west, about the Bechuanaland Protectorate and South-West Africa borders, there is a wide transitional zone. A striking association, however, is that of the shallow tufaceous limestone soil and the Kalahari thorn scrub.

It must be noted that Pole Evans' classification is not based entirely on dominance of vegetation. His Thorn Country in Bechuanaland Protectorate is listed under Desert, whereas Thorn Country, for example, in Zululand falls under Grassland. This point is mentioned for, occurring in the Savanna Vegetation Area, in the north-eastern part of the Bamangwato Native Reserve and elsewhere, are large areas of Thorn Country widely separated from his main Kalahari Thorn Area. It would therefore seem more appropriate to adopt at any rate for the *Middle Kalahari*, the scheme of Henekel (1931), who, for Southern Rhodesia, considers Thorn Country as a unit of Woodland, irrespective of where it occurs. Kalahari Thorn Country will therefore find a place under Open Tree Country, although this again is apt to mislead, for in some areas, the actual woodland vegetation may be scrub, for example, east of the Okovango Delta, and in others, the formation may be forest which is not "open," e.g. *Acacia giraffae* forest from Gomare southwards. Whatever classification is adopted has points of difficulty and the position must be judged on general lines.

The magisterial districts of Ngamiland and Chobe may be divided botanically into:—

(A) Grassland, and (B) Woodland.

(A) The former occurs (*a*) as Marsh Grassland where inundation takes place or has taken place within recent times (see Figs. 1 and 3); and (*b*) in Open Tree Country or Savanna, i.e. grass country "with isolated trees or trees growing in denser formation" (Pole Evans). Grass in this region (Bushveld) is evident chiefly during the rainy season when it grows luxuriantly and is therefore most conspicuous. It is, however, intended to deal with this type under Woodland, for in the Middle Kalahari trees generally predominate.

(B) The latter type may be divided into (*a*) Closed Fringing Forest of evergreen type lining the flood channels (see Fig. 2), or occurring as "islands" in the Marsh Grassland (see Fig. 4), and (*b*) Open Tree Country of deciduous character situated away from the flood channels above high water level. The term "open" has been qualified above, but it must be stressed that generally this type of Woodland is more open than Fringing Forest.

GENERAL DESCRIPTION OF EACH TYPE.

(A) GRASSLAND.

(*a*) *Marsh*.—This term is selected because of a definite association, whether now or formerly, with flooding from either the Okovango or Chobe River Systems. As indicated under environmental factors, water is the chief limiting agent in the Middle Kalahari, not only because of the water *per se*, but also owing to the *winter* floods having brought down for centuries a black soil, on the floor of which flourish succulent herbs, e.g. *Gramineae*, *Cyperaceae* and *Polygonum* sp.

This type of Grassland is generally free from trees although breaking the meadow-like continuity in some places there may be "islands" of dense trees. Indeed, during the flood season these groups of trees, growing on ancient termite mounds, are in truth islands. Again there is an absence of bulbous plants, e.g. *Liliaceae*, and prostrates, e.g. *Tribulus terrestris*, or *Harpagophytum* sp. and members of the family *Cucurbitaceae*, etc.

Grassland, such as described above, is to be found along the Okovango River System from Mahembo south (and also in the Caprivi Strip), especially after it divides to form the Delta. To a less degree is grassland developed in connection with the Chobe River, although west of Kachikau (and the eastern part of the Caprivi Strip) is to be found more or less pure grassland. In fact the latter district was formerly the favourite grazing ground of the cattle belonging to the Barotse royal house. Stigand's (1923) map gives the best idea of the extent of country in Ngamiland inundated during flood seasons.

It is clear then that Marsh Grassland may vary from a permanent swamp containing *Phragmites communis*, *Typha australis* and *Cyperus* spp. to flood channels which are only occasionally submerged. In either case grass or meadow land (*Diplachne* sp. is common) occurs along the ramifications of the Okovango and Chobe River Systems which form a maze through the Open Tree Country. There is no more beautiful sight, especially to one who has just traversed the surrounding grey sandy country, than a view along the Okovango

Delta. Here one sees a placid creek or lagoon leading from a main branch, with papyrus lined banks. At a shelving beach are pelican and flamingo while on the lily (*Nymphaea stellata*) covered water swim innumerable duck, geese and other water birds. In the background is a wall of dark green forest, above the blue sky and around silence. There, further on, the country is less wooded and one observes a broad expanse of green swamp country, with islands of palms (*Hyphaene* sp.) dotted about, their tall erect stems being conspicuous for miles around. Not a soul in sight, but perhaps a timid Masarwa is within a stone's throw so dense is the fringing forest. This country is indeed a paradise, but for how long?

A generation or so ago similar country in the south-west is now desert, the swamp floors are covered in summer not by water from the parent branch, known here as the Taoge River, but by rainstorms. Around are desert grasses, e.g. *Aristida* sp., *Urochloa* sp., *Eragrostis* sp., and perhaps a carpet of *Tribulus terrestris*, and the fringe of former evergreen bush can be traced by the dead trunks of *Combretum* sp., *Phoenix reclinata* or *Hyphaene* sp., and a straggling *Lonchocarpus* sp., *Kigelia* sp., or *Aleurites* sp. Still more striking is the relentless encroachment by *Acacia* spp., especially *A. giraffae* and *A.* sp. ("Mokwa"). Stigand's (1923) map indicates desiccation in the south-west and south, but not to the east of the Delta. By taking the cattle road from Puluhele to Tsotsoroga Pan, these changes may be observed *en route*, but the dominant woodland is not altogether *Acacia* but scrub *Copaifera mopane*, *Terminalia* sp. and *Grewia* sp.

About the perimeter of the Delta, where Glossina have not yet penetrated, are native habitations with their fish traps and cattle kraals. Whereas cattle graze unrestricted for the greater part of the year, during the early summer suitable areas of the grassland, especially along the Lower Boro River, are cultivated, in spite of some risk of flooding by local rainstorms (Kurube, Taoge River, December, 1930), whereas in the sandy Open Tree Country every drop is absorbed. The extent of winter flooding is shown not only by the type of vegetation but also by the presence of numerous shells of the genera *Lanistes*, *Pila* (snails) and *Caclatura* (mussel)*. Near populous centres, e.g. Maun, the bleaching bones of cattle demonstrate to what degree deaths may occur through hogging or starvation or both. As a matter of fact one may estimate fairly accurately the whereabouts of Glossina by the state of grazing in the flood channels. If the grass is tall and untouched it is clear that Glossina are present in the vicinity. It must also be remembered that cultivation and overgrazing tend to alter the constituent grasses, *Cynodon dactylon* being a common sequel, and that circumstances generally are favourable to infection of stock by internal parasites, e.g. through early summer grazing along the flood channels.

Finally it must be noted that the Marsh Grassland, where Glossina are absent, represents the favourite grazing ground of the ruling tribe, the Batawana; but under a properly controlled system of pasture management Open Tree Country could be used in summer and autumn (when the pans contained water) and swamp grazing would be restricted to the winter and spring months.

* Kindly identified by Mr. V. Fitzsimons, Transvaal Museum, Pretoria.

(b) *Open Tree Country*.—The sandy country characterising the mixed grass and tree region is exceedingly dry, in fact desert-like. The grassy covering is therefore best seen after the summer rains when in depressions, such as pans, patches of pure grassland may be observed, but generally it occurs as tufts dotted about among the scrub and trees. The grass, which is quickly maturing, varies greatly in grazing values, “*nphaga*” (*Panicum maximum*) being excellent towards the end of the summer, whereas the coarser “*molikang-wetsi*” (*Pennisetum ciliare* and *P. cenchroides*) and “*motenyane*” (*Schmidtia bulbosa*) last well through the winter. The most striking fact, however, especially in the well-stocked southern area is the dominance of the shade-loving “*poke*” (*Urochloa helopus*), an excellent fodder.

At present, apart from the Damaras, who as intruders are not encouraged to graze their cattle along the Marsh Grasslands, very little use is made of this rich pastoral country. Later, when wells are sunk, it will be rightly considered as ideal ranching country.

As tree growth, including scrub, predominates, further remarks will be made under Woodland. It will therefore suffice if the position regarding Grassland is summarized thus: whereas the Marsh Grassland generally occurs as a carpet of pure succulent meadow, the sandy Open Tree Country is characterized by uneven growth of a xerophilous, and more temporary, nature.

(B) WOODLAND.

(a) *Closed Fringing Forest*.—Separating the two forms of Grassland described above is usually a belt of dense water-loving forest of evergreen type. As one approaches the periphery of the Delta and water is scarce, so does the Closed Fringing Forest become less evident, a good example being the country along the Maun-Toten road. The chief species contributing to this evergreen fringe are *Ficus* sp., *Phoenix reclinata*, *Hyphaene* sp., *Aleurites* sp., *Syzygium guiniense*, *Strychnos* sp., *Combretum imberbe*, *Lonchocarpus capassa*, *Kigelia pinnata*, *Trichelia* sp., *Acacia* spp., and most striking of all “*mokuchon*” (*Diospyros mespiliformis*), the tallest, and “*mot-saodi*”*, the shadiest tree of the Okovango Delta. Both the last mentioned have edible fruits.

Dotted about the Marsh Grassland are often to be observed “*islands*” (usually old termite mounds) densely covered with evergreen trees. Beneath are frequently vines and shrubs, e.g. *Gymnosporia* sp., *Asparagus* sp. or *Grewia* sp., but grass is usually absent.

Country such as described above is of particular value to *Glossina* in the winter and spring months, and in aerial photography, especially during this season, is a conspicuous feature of the landscape. In extent, however, it is of relatively minor importance for covering over 90 per cent. of Ngamiland and Chobe is Open Tree Country.

(b) *Open Tree Country*.—It is possible to subdivide this into two main groups, viz:—(1) Thorn (see Fig. 5); and (2) Non-Thorn (see Figs. 7-11), but whether it is justifiable to further classify the Non-Thorn group is a matter for thought. Henkel (1931), in

* “*Motsaodi*” is *Garcinia livingstonii*.

describing the Open Tree Country units of Southern Rhodesia, has relied much on altitude. Now, in Ngamiland and Chobe, altitude is of minor importance for it shows little variation. Further, it is remarkable how uniform in distribution is not only the sand country flora, but also the Closed Fringing Forest, provided water is available. A noteworthy exception regarding the latter is the "moku-chon" which seems to be restricted chiefly to the country west of longitude 23° provided water is in sufficient quantity.

After consideration, it seems permissible to further subdivide (2) Non-Thorn Country into three main groups, viz:—(i) "Mopani", *Copaifera mopane*; (ii) "Mosheshe", *Burkea africana*, and (iii) "Mogonono", *Terminalia sericea*, an opinion in fact indicated by Stigand (1923).

Regarding (1) *Thorn*, this includes chiefly *Acacia* spp., varying from *A. giraffae* in forest formation west of the Taoge River and around Lake Ngami to the scrub-like *Dichrostachys* spp. (see Fig. 6). It occupies mainly the southern part of Ngamiland as has already been stated, and a little south of latitude 20° approximately indicates its northern limit. At the longitude passing through Sihitwa on Lake Ngami along the above line of latitude, thorn country shows a marked extension to the north along the Taoge River until a few miles north of Gomare and thence to the Tshodilo Hills. While the north-eastern limit is well marked, the western boundary of this indentation is apparently not so clear, there being a marked transitional zone between this and the *Terminalia* sp. country still further to the west.

In the Non-Thorn areas, *Acacia* spp. also occur but generally are not dominant. They are characteristic in places where desiccation is occurring, e.g. along the Mogogelo and Mababe Rivers and around the Mababe Depression and south of the Sici-Kachikau swamp region.

To the east of the Delta, apparently beyond the seepage limit, typical Kalahari Thorn Country (Pole Evans) is to be seen along the cattle road north and south of Sakobs Pan. It was this country through which Livingstone (1857) travelled during the winter of 1850 en route to the Chobe River, for he describes the scene as dreary and "the only vegetation was a low scrub in deep sand". While *Acacia* sp. is common around Sakobs Pan, a few miles north is a vast area of *Dichrostachys* sp., hundreds of acres of which in January, 1931, were killed, presumably by fire. At that time, however, owing to copious rains the Open Tree Country grasses were luxuriant. It might be added that the water level in this vicinity is at least 100 feet deep.

It is convenient to refer here to the succession which is taking place from Swamp Country (including Marsh Grassland and Closed Fringing Forest) to the Thorn type of Open Forest Country.

Stigand (1923) has partly referred to this phenomenon when he describes the replacement of swamp "with short quickgrass . . . in about five years". This grass is *Cynodon dactylon*, the spread of which is encouraged by over-stocking, there being thousands of head of native cattle in the southern part of the country.

Commencing with a swamp or *permanent* flood channel (see Fig. 12) containing papyrus, *Typha* sp. and *Phragmites* sp. and bordered by *Phoenix reclinata*, *Hyphaene* sp., *Ficus* sp., *Combretum imberbe*, *Lonchocarpus capassa*, etc., the second stage is *annual* flooding during the winter. Papyrus is the first plant to disappear, and whereas only the edge of the swamp was formerly cultivated, the entire floor is now ploughed about November and the rich black soil produces a fine crop of maize (see Fig. 13). There is of course a risk of the mealies being "drowned" by heavy summer rains. In the course of time only *occasional* flooding, the third stage, takes place in the winter and there follow marked alterations in the botany of the area. What stragglers have survived owing to annual inundation, e.g. *Phragmites* sp., now perish, and the fringing trees gradually die and over a period of 15-25 years the appearance of the former swamp completely changed (see Fig. 14). Not only has *Cynodon dactylon* replaced the original members of the family *Cyperaceae*, but cultivation is only possible after the summer rains. Whereas the floor was formerly black and hard, the soil becomes sandier and more porous. With overgrazing by cattle, the spread of *Urochloa hieopus* has been encouraged. In the meantime not only have desert grasses, e.g. *Aristida* sp. and *Eragrostis* sp. and trees, e.g. *Acacia* sp., "mogotlo" (*A. giraffae*), "mokwa", and "moshu" (*A. litakumensis*) become well established about the periphery of the area, but cultivation ceases, the natives preferring to be nearer the receding swamp country. Encroachment chiefly by *Acacia giraffae* now proceeds unhindered and the fourth or *final* stage of complete replacement by "thorn" occurs. The soil becomes so porous that a pan only forms during the wettest of summers, and associated with the desertion by the natives, is the spread of game and perhaps the extension of *Glossina*, the curse of the Okovango Delta. In a phrase then, it may be said that what was a lake or swamp (e.g. Lake Ngami) will in time become a thorn forest (e.g. Ngami forest of the future). See Fig. 15.

Regarding the (2) Non-Thorn group (i) "*Mopani*" is the most important of the three sub-divisions, especially within the Delta region (in the sand country above flood level) and south of the Chobe River.

Although a patch of "mopani" was encountered between N'kemi and Namasseri, west of the Okovango River, yet it is understood it occurs still further to the west, viz. in South-West Africa Protectorate. The southern limit is well marked, viz. a little south of latitude 20°.

On the journey along the cattle road from Puluhele to Tsotsoroga Pan striking differences were noticed in form and distribution. The tree form ceased abruptly east of Riley's Road as far almost as Xana Pan, and where "mopani" occurred along the cattle road, it was usually as scrub. Between Matsalele ya Tou and Tsotsoroga Pan (where much rain water collects in pools) the "mopani" was actually forest-like with little undergrowth excepting "mopani" itself. After the summer rains grass is generally more conspicuous than "mopani" where it occurs as coppice growth.

Associated with "mopani" are many trees, e.g. *Terminalia* sp., *Combretum* spp., *Kirkia* sp., *Pterocarpus* sp. and shrubs, e.g. *Grevia* spp., *Rhus* spp., *Royena* sp., etc., the most characteristic, however, being *Adansonia digitata*, which is particularly common along the Okovango River, especially north of Andara.

(ii) "Mosheshe" is dominant in the north-west corner of Ngamiland, along both banks of the Okovango River. Extensive areas, however, are to be found in Chobe magisterial district, especially south-west of the Goha Hills. West of the Okovango River between Andara and Gomare *Dichapetalum* sp. is widely distributed. It is about knee-high and bears much fruit. Common undergrowth is provided by *Bauhinia* spp. and *Baphia* of which there appear to be two species, and frequent trees are *Peltophorum africanum* and *Acacia pallens*.

(iii) "Mogonono" is definitely the type which is best able to withstand desert conditions. Not only is it dominant in the arid sandy country west of the Okovango Delta, but when it occurs elsewhere, e.g. in the "Mopani" region south of the Chobe River, it seems to flourish on the dry sandy ridges.

An interesting phenomenon was observed north and south of Ranta, west of the Taoge River. Here thousands of acres of *Terminalia sericea* had been "drowned" by excessive flooding of the Taoge River in the winter of 1927. During the following summer the growth of grass had consequently become unduly rank and on being burnt, a tremendous extent of "mogonono" country had been destroyed. This fact at once suggests the need for systematic research by forest workers in regard to the destruction of trees in *Glossina* country.

Regarding the Non-Thorn types, least is known of the distribution of "mogonono" as a dominant. It was encountered in large numbers between N'Kemi and Gomare, but according to Stigand (1923), it is dominant as scrub forest west of longitude 22°.

Finally, it should be stressed that although more than three-quarters of the plants listed in Part II belong to the Open Tree Country flora, yet intensive study of the swamps would reveal a wealth of material (algae and aquatics generally) much undoubtedly new.

POISONOUS PLANTS.

Plants known to be poisonons (based mainly on observations carried out at Onderstepoort, Pretoria), are:—*Abrus precatorius*, *Aselepias fruticosa*, *Cucumis africanus*, *Dichapetalum cymosum*, *Geigeria zeyheri*, *Ricinus communis*, *Solanum panduraciforme*, *Tribulus terrestris* and *Urginea altissima*.

The *D. cymosum* was knee-high and bore much sound fruit. The *T. terrestris* appeared to include several varieties.

In addition, several grasses known to be cyanogenetic were encountered as follows:—*Eragrostis superba*, *E. lehmanniana*, *Sporobolus fimbriatus*, *Aristida uniplumis*, *A. congesta* and *Cynodon dactylon* (Henrici 1926).

Plants suspected of being toxic are: *Dipcadi* sp., *Gloriosa* sp., *Mundulca suberosa*, *Aleurites* sp. ("Mutsibi", a fish poison), *Malva parviflora* and *Adenia* sp. Green shoots of *Phragmites communis* are also believed by natives to be dangerous to stock, possibly owing to the causation of tympanites.

SUMMARY.

Not only have the floral regions been outlined, but other facts concerning Animal Husbandry in this future ranching area have been given as follows:—(1) The Map itself shows the two Glossina areas of the Okovango Delta and of the south bank of the Chohe River respectively. (2) The value of the various grazing areas has been indicated. (3) Poisonous plants observed during a trip around the Delta have been noted. (4) The fact that scrub occurs east of the Thamalakane River is an explanation for the absence of Glossina in pre-Rinderpest days between the above river and the present Bechuanaland Protectorate—Southern Rhodesia border (old Hunters Road). Finally (5) the dangers of desiccation have been emphasised.

APPRECIATION.

It is a pleasure to acknowledge the assistance received from Dr. I. B. Pole Evans, Chief, Division of Plant Industry. Dr. P. J. du Toit, Director of Veterinary Services, kindly allowed the typing and photographic work to be done at Onderstepoort, and finally my colleague, Dr. D. G. Steyn, gave me the benefit of his wide experience on poisonous plants.

REFERENCES.

- BEWS, J. W. (1918). *The Grasses and Grasslands of South Africa*. P. Davis & Sons Ltd., Maritzburg, p. 100.
- CURSON, H. H. (1932). Distribution of Glossina in the Bechuanaland Protectorate. This Report.
- DU TOIT, A. L. (1927). The Kalahari and some of its problems. *S. Afr. Jl. Sc.*, Vol. 24, p. 88.
- DU TOIT, A. L. (1926). *Report of the Kalahari Reconnaissance of 1925*. Govt. Printing and Stationery Office, Pretoria.
- HENRICI, M. (1926). Preliminary Report upon the Occurrence of Hydrocyanic Acid in the Grasses of Bechuanaland. *11th & 12th Rpt. Dir. Vety. Educ. & Research*. p. 495.
- HENKEL, J. S. (1931). Types of Vegetation in Southern Rhodesia. *Proc. Rhodesia Sc. Assn.*, Vol 30, Reprint.
- LIVINGSTONE, D. (1857). *Missionary Travels and Researches in South Africa*. John Murray, London. p. 79.
- PASSARGE, S. (1904). *Die Kalahari*. Dietrich Reimer (Ernst Vohsen). Berlin.
- POLE EVANS, I. B. (1931). The Vegetation of South Africa. *Official Year Book of the Union of South Afr.*, No. 12, 1929-1930. Govt. Printing & Stationery Office, Pretoria. p. 25.
- STIGAND, A. G. (1923). Ngamiland. *Geog. Jl.*, Vol. 62, pp. 401-419., (Dec.) Two large scale maps dated 1922 were published in addition.





Fig. 1.

Chobe River near Sanazambo Pan. Note *Phragmites communis* which during the winter floods is entirely inundated. Appearing on the right is *Phoenix reclinata* belonging to the Closed Fringing Forest type of vegetation.—10.11.30.



Fig. 2.

Typical view of Closed Fringing Forest on right bank of Chobe River near Sanazambo. Trees are *Acacia* spp. and *Phoenix reclinata*. In foreground is Marsh Grassland—10.11.30.



Fig. 3.

Country west of Kachikau formerly flooded annually by Chobe River, and now only occasionally under water. Growth of grass due to summer rain. Note in distance encroaching *Acacia* spp.—25.1.31.



Fig. 4.

Closed Fringing Forest in "island" formation between Gonotsoga and Sanxanxa—8.12.30. (Photo Dr. Hale Carpenter).



Fig. 5.

"Thorn" Country in bed of Nghabe River, Totten. The *Combretum imberbe* has been dead for over 40 years.



Fig. 6.

Dichrostachys sp. along the cattle road to Tsotsoroga Pan from Maun. A view taken 15 miles north of Sakobs Pan. Note dead tree trunks and type of grass—Taken from wagon 19.1.31.



Fig. 7.

Tsotsoroga Pan in the heart of the Mopani country during the dry season. The fallen tree is *Lonchocarpus capassa* and beside it is *Combretum imberbe*—12.11.30.



Fig. 8.

The same view as Fig. 7 taken after the rains. The luxuriant growth of grass almost obscures the water—23.1.31.



Fig. 9.

In the Mopani scrub along the cattle road from Maun to Tsotsoroga Pan. Fourteen miles north of Puluhele—17.1.31.



Fig. 10.

Abrupt transition from tree mopani to scrub mopani four miles south of Xana Pan—20.1.31.



Fig. 11.

Dead *Terminalia* north of Ranta along the west bank of Taoge River. Thousands of acres of trees were "drowned" by excessive flooding in 1927 and as a result of fire the following summer, their destruction was completed. Note normal *Terminalia* forest towards the left background—24.12.30.



Fig. 12.

A permanent flood channel, the Thamalakane River, 4 miles south of Maun. Note reeds in the river bed, overgrazing of meadow-like right bank, and Closed Fringing Forest on left bank—14.1.31.



Fig. 13.

Second stage in transformation of swamp to Thorn forest. Annual winter flooding of channels makes it possible to plant mealies in spring. A scene near Depeng's Village, north of Tsau. Note wilted plants at edge of field—29.12.30.



Fig. 14.

Third stage (see Figs. 12 & 13), a view near Cencu Village, but on right bank of Taoge River. Area is only occasionally inundated. Note growth of young *A. giraffae*—30.12.30.



Fig. 15.

The final phase. A view on west side of Lake Ngami which formerly covered by grass is now a forest of *A. giraffae*—1.1.31.

A Peculiar Case of Traumatism affecting the Metatarsal Bones.

By N. T. v. d. LINDE, Veterinary Student, Onderstepoort.

A MARE (horse), 2 years old (unbroken), was running in a paddock on a farm in Griqualand West. In some way or other the near hind leg was caught in a wire fence, and the animal was noticed to be lame



three days later. About three weeks later a native shepherd informed the owner that the leg was tremendously swollen and that the animal could hardly walk. The mare was accordingly cast and examined. The leg was found to have an enormous swelling of a very firm consistence about the middle of the metatarsus. After another three weeks the animal was destroyed and the near metatarsus was removed and cleaned. (See figure.)

At the junction of the distal and middle thirds of the metatarsus, a piece of No. 17 Eland fencing wire was attached around the large metatarsal bone and the distal ends of the two small splint bones. This caused inflammation and proliferative changes due to the continual irritation. An ossifying periostitis and osteitis with resulting osteoporosis and osteosclerosis set in around the large metatarsus, except at the plantar space. In this way a bony canal of newly formed bone was found around the wire, which very nearly cut the metatarsus down to the medullary cavity. The distal extremities of the second and fourth metatarsal bones were also involved, a large exostosis being formed in each case. Below the circular canal newly formed spinous processes had grown downwards and slightly outwards. On the dorsal surface there were several small canals opening to the outside.

It is remarkable that this enormous growth of bone had occurred after a period of only six weeks.

Anatomical Studies, No. 28: Hypospadias in a Merino Ram.

By G. C. VAN DRIMMELEN and A. R. THIEL, Veterinary Students, Onderstepoort.

On 16.3.31 a Merino ram (D.O.B. 26157) about 18 months of age was submitted to us for dissection. The history was that the animal had been received from Grootfontein School of Agriculture on 6.3.30 along with 20 other ewes for nutrition experiment! It was, however, not placed in that experiment. Further, it was believed to be a hermaphrodite.



Fig. 1.

On external examination the following anomalies were observed: (a) the scrotum was divided into two distinct sacs (see Fig. 1); (b) the "urethral process" was 1 cm. in length but possessed no lumen; (c) the penis and prepuce were smaller than normal; (d) a marked shallow groove extended longitudinally downwards and forwards from the anus (in the medial line) to within 8 cm. of the orifice of the prepuce; (e) in the bottom of the groove about 1.5 cm. below the anus was a slit-like opening which proved to be the external orifice of the urethra. This was pouch-like and bore some resemblance to a vulva. Its length was 3 cm.

On internal examination of the urogenital system it was clear that: (1) the testicles were smaller than usual, especially the left; (2) the bladder was poorly developed; (3) the urethra showed no



Fig. 2.

extension beyond the crus penis; (4) the corpus cavernosum urethrae was absent; and (5) the urethra being pelvic, was short and like that of a ewe. In the accompanying figure (Fig. 2) a probe has been inserted in the urethra.

HISTOLOGY.

The right testicle showed spermatozoa, as did the epididymis, whereas the seminiferous tubules of the left gland were atrophied. In both cases there was fatty degeneration of the basal epithelial layer (spermatogonia), especially of the left testis. (See Fig. 3.)

Fig. 3A.



Fig. 3B.



EMBRYOLOGY.

It is clear that since there was no combination of male and female sexual organs that the sheep was not a hermaphrodite, but an example of hypospadias, in fact an extreme form where even the scrotum has become involved. The anomaly is teratological in nature, being due to incomplete fusion of the urethral folds along the under surface of the genital tubercle. The groove described along the prepuce is out of place, its true position being the under surface of the corpus cavernosum penis.

Since writing the above note, we have encountered two further cases of hypospadias in the Merino, one in a ram of $2\frac{1}{2}$ years (D.O.B. 30996), and the second in a ram of 4 years (D.O.B. 30997). (See File 141/1605.) The anomalies in both cases were very similar to those described for sheep 26157, the only difference being in Sheep 30997, where the external urethral orifice was approximately 10 cm. from the anus.

Anatomical Studies, No. 29: A Further Note on Free-Martinism.

By H. H. CURSON, F.R.C.V.S., Dr. Med. Vet., Veterinary Research
Officer, Onderstepoort.

INTRODUCTION.

In a previous study (Curson 1930) details were given concerning two free-martins. It was further stated that particulars regarding twinning in cattle in South Africa were difficult to obtain. Since then a little information has been furnished by the Schools of Agriculture. This will be given in the following note, with details, regarding additional cases of free-martinism.

TWINNING.

The attached statement in tabular form represents the information referred to above.

Supplementary information is also available regarding twins of different sexes as follows:

The Principal (Fisher, J.), School of Agriculture, Cedara, reports (his 9/1/2 of 22.4.29) that a Friesland cow D.O.A. 13C had a set of twins of which the male was sterile. He adds, "we have had experience of several heifers born twin with bull being sterile".

The Head, Department of Animal Husbandry (Prof. Reimers) Stellenbosch-Elsenburg College of Agriculture, states (his S. 4/13 of 24.4.29), "it has always been the practice to sell the female to the butcher. Males from twins have been sold many times and we have never received any complaint about their fertility". In the same letter reference is made to "a grade Friesland cow which, served by a Jersey bull, had given birth, 22.2.20, to four calves—three heifers and one bull". The bull proved to be fertile and of the three heifers "though one came often in season", all proved infertile and were sold to the butcher. Post-mortem examination by Mr. B. S. Parkin, M.R.C.V.S., revealed arrested development of the internal genitalia characteristic of free-martinism.

		Elsenburg.		Stellenbosch.		Potchefstroom.	Glen.	Groetfontein.	Cedara.	
		Fries.		Fries.	Jersey.				Fries.	Ayrshire.
(a)	Year.....	—		—	—	1927-29	1921-29	1927-29	1923-29	
	Cows.....	262		115	309	—	299	—	33	60
	Calves.....	11 sets = 4.2%		5 sets = 4.3%	5 sets = 1.6%	—	4 sets = 1.3%	0 sets = 0%	124 0 sets = 0%	573 5 sets = 0.9%
(b)	Twins.....					1.8%				
	Sets of twins	2 = 18.1%		2 = 40%	1 = 20%	—	1 = 25%	—	—	1 = 20%
	Of the sets of twins, the percentage where both were female									
(c)	Sets of twins	1 = 9.1%		2 = 40%	2 = 40%	—	1 = 25%	—	—	1 = 20%
	Of the sets of twins, the percentage where both were male									
(d)	Sets of twins	1		—	1	All sets See "Heifer" 3590 (Curson 1930)	2 = 50% See "Heifer" 2971 (Curson 1930)	—	—	2
	Of the sets of twins, the percentage where 1 was male and other female									
(e)	Sets of twins	7 = 63.6%		1 = 20%	1 = 20%	—	—	—	—	1 = 20%
	Of the sets of twins, the percentage where no reliable data available									

It is regretted that under (d) it was impossible to make a distinction between (i) normal male and normal female; (ii) normal male and free-martin; (iii) sterile male and normal female; and (iv) sterile male and free-martin, inasmuch as the calves were rarely allowed to reach maturity. Births also include premature births.

The Lecturer in Animal Husbandry, Glen School of Agriculture (his 277 of 22.4.29), reports "In my personal experience I have not known a single case of a female twin of twins of opposite sexes to calve. In some cases the periods of oestrus were very irregular, while in others no signs of oestrus were ever noticed, and these heifers were always very narrow between the pin-bones. A female twin of twins of opposite sexes which had calved was shown to me at the Central Show, Bloemfontein . . ."

FOUR CASES OF FREE-MARTINISM.

(a) *Friesland "Heifer" 3627 (Path. Spec. 10,731).*

The above animal, aged $2\frac{1}{2}$ years at slaughter, arrived from A. H. Coetzee, Esq., Kirkwood, C.P., on 14.8.29 and after passing through several experiments was killed on 7.6.30. No information is available as to whether "she" ever manifested oestrus, but from an inspection of her ovaries it would appear that such was not the case. (See Figs. 1 and 2.)

(b) *Sussex-Afrikander "Heifer" 3636 (Path. Spec. 10,729).*

The above animal, born 15.12.27, arrived from the School of Agriculture, Potchefstroom, on 22nd October, 1929, and after passing through trypanosomiasis experiments was killed on 22nd September, 1930. (See Figs. 3 and 4.) Enquiries from the Principal, School of Agriculture, Potchefstroom, indicate that "she" was not under observation and therefore, whether oestrus occurred or not is not known. Judging from the appearance of "her" ovaries it is not likely such occurred.

(c) *Friesland "Heifer" 4226 (Path. No. 11,401).*

The above animal was one of a set of twins born towards the end of January, 1930, at Mariendahl, near Stellenbosch, and kindly sent to Onderstepoort, by Prof. Reimers, Head, Department of Animal Husbandry, on 9.9.30. "She" was never observed to show oestrus, which fact can be readily appreciated when the internal genitalia are examined (see Fig. 5). Killed on 23.3.31 for Teratology class.

(d) *Red Poll "Heifer" calf 3359 (Path. No. 11,341).*

The "heifer" was one of a set of twins born at Arnoedsvlakte, P.O. Vryburg. On "her" death on 8.3.31, the internal genitalia were thoughtfully sent to Onderstepoort by Mr. J. H. R. Bisschop (Lab. Spec. 8044). (See Fig. 6.)



Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.



Fig. 5.



Fig. 6.

Anatomical Studies, No. 30: On Two Cases of Atresia Ani.

By H. H. CURSON, F.R.C.V.S., Dr.Med.Vet., Veterinary Research Officer, Onderstepoort.

INTRODUCTION.

IN this note it is intended to describe (i) a case of atresia ani, with the rectum opening into the vagina, Heifer 3670, and (ii) a case of atresia ani urethralis, Bull calf 1227, which conditions, especially the latter, are not frequently met with.

HEIFER 3670.

Thanks to the generosity of Mr. J. Robertson, of Hluhluwe, Zululand, the above animal, a red heifer, born early in 1929, was handed to Mr. J. L. Dickson, B.V.Sc., Government Veterinary Officer, Nongoma, who sent her to Onderstepoort during the middle of November of 1929. As is clearly shown in Fig. 1, there was no anal aperture, the urogenital opening serving as a cloaca as illustrated by Fig. 2. (See File 142/1371.)



Fig. 1.—Heifer 3670.
(External view.)

The heifer was killed on 4.4.1930 which fact clearly indicates that such an anomaly will not interfere with life. Bradley (1899) in a paper on "Atresia ani" gives diagrammatic representations of the various forms of the anomaly.

Embryologically, the anomaly is due to failure of complete development of the uro-rectal fold which normally separates the rectum and urogenital sinus (Bailey and Miller 1927).



Fig. 2.—Heifer 3670.
(Internal view.)

The specimen represented in Fig. 2 has been placed in the Teratological Museum under Pathology No. 11348.

BULL CALF 1227.

The above animal was purchased from a native on 31.7.1925, killed the same day and kept in the preserving tank until 1.2.1930. It would appear that the calf was born on 30.7.1925, and the owner, realizing that the condition was incompatible with life, decided to bring it to the Laboratory.



Fig. 3.—Bull calf 1227.



Fig. 4.—Bull calf 1227.
(Note absence of anus.)

Fig. 3, taken on 31.7.1925, shows the animal when alive and the presence of an accompanying anomaly, acoceyegeus, which is uncommon. Fig. 4, natural size, shows the hairless anal region which lacks an orifice.

On dissection it was found (Fig. 5) that the rectum ended blindly (atresia recti), but ventrally it communicated with the urethra through an opening approximately 0.25 cm. in diameter (see probe).

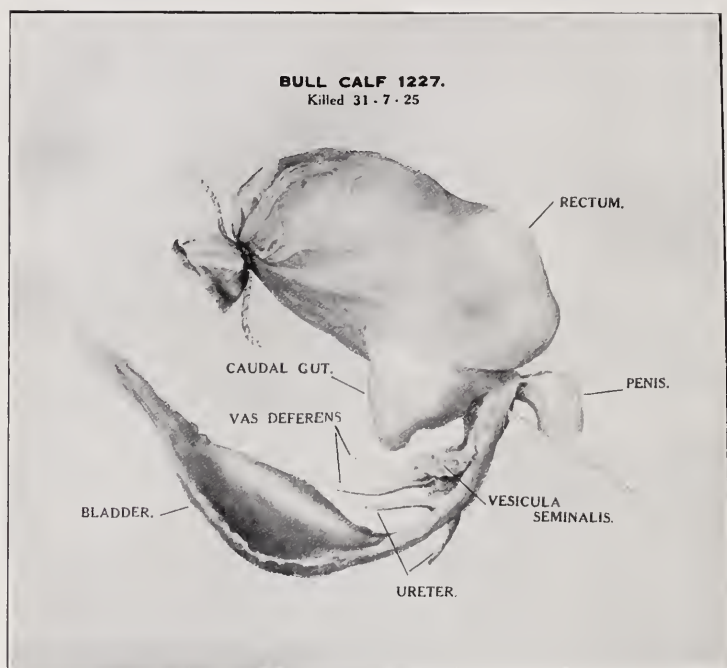


Fig. 5.—Bull calf 1227.
(Internal view of terminal
region of alimentary tract
and commencing portion of
urinary tract.)

Embryologically the anomaly is due to an arrest in development of the uro-rectal fold. The Museum number is Pathology No. 5401.

REFERENCES.

- BRADLEY, O. C. (1899). Atresia ani. *Vet. Jl.*, p. 324, May.
BAILEY, F. R., and MILLER, A.M. (1927). *Text-book of Embryology*.
Wm. Wood & Co., New York, p. 326.

Anatomical Studies, No. 31 : On Two Cases of Acardiacus.

By H. H. CURSON, F.R.C.V.S., Dr. Med. Vet., Veterinary Research Officer, Onderstepoort.

SINCE the above condition is not common, a short description of two cases, (*a*) goat, and (*b*) cow, is appended.

ACARDIACUS COMPLETUS IN A GOAT.

Although in domesticated animals the only definite evidence of true twinning is where one body is normal and the other has become arrested in development, constituting an *Acardius* (or *Acardiacus*), frequently an *Acardius amorphus* (*globosus*); yet it is possible in the above case, where sex was identical (both male) that true twinning had occurred and that blood supply had been sufficient to form an *Acardius completus*, i.e. fairly normal foetus. There is, of course, the possibility that (*a*) twinning had resulted from the extrusion of two ova during ovulation (either from one or each ovary) i.e. superfecundation, or (*b*) twinning had followed superfoetation.

In any case in the sheep and goat twinning is usually associated with double ovulation and non-anastomosis of the placental blood vessels.

As will be seen in Fig. 1 there is a decided difference between the normal twin and the malformation, both of which were removed from the same uterus and entered in the Museum Collection under Path. No. 2620 of 28.5.23. No details are available with regard to placentation.

While one animal had developed normally and was approximately 5 months of age, the condition of the anomaly resembled that of a 12-14 week old foetus, viz.: there was no hairy covering, and only the eyelashes and tactile hairs were present. Whereas the normal foetus weighed 1.3 Kg. and measured 33 cm. the anomaly weighed 0.9 Kg. and measured 28 cm.

It is, however, certain that arrest in development had commenced very early in embryonic life, for as shown in Fig. 1 the nasal region was deformed. Further the right eyeball was absent (monophthalmia).

ACARDIACUS AMORPHUS (*Mola cruenta*) IN A COW.

The above malformation is generally encountered in cattle practice and I am indebted to Mr. A. M. Diesel, M.R.C.V.S., formerly Government Veterinary Officer at Bethlehem, Orange Free State, for the specimen (see Fig. 2) and photograph of the cow and normal individual (see Fig. 3) (File 141/1904).

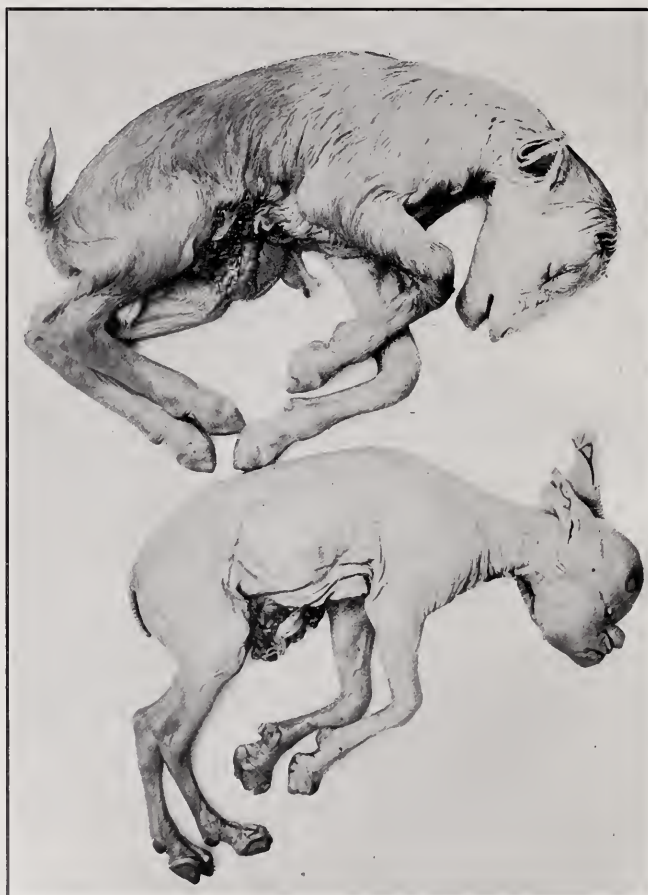


Fig. 1.

The structure in question, showing an umbilical cord and the typical black and white markings of a Friesland "came away along with a full time bull calf from a pedigree Friesland cow". On section of the soft fleshy mass nothing distinctive was observed (Path. No. 8883).

As mentioned above, it is in such a case as this that the occurrence of true twinning is accepted for domesticated animals.



Fig. 2.



Fig. 3.

Anatomical Studies, No. 32: Atresia Ani with Rectum Opening into Vagina in a Kitten.

By W. D. MALHERBE, Veterinary Student, Onderstepoort.

A FEMALE MAUX kitten of 6 weeks of age was brought by me to Onderstepoort on 2.5.30 with the history that she had not passed any large quantity of faeces since her birth, and that the animal appeared to have only one " passage ".

On examination, the following facts were noted:—

- (1) The animal appeared to be in constant distress and continually made noises to indicate such distress. It often stood in a position of tenesmus.
- (2) Only at intervals was it able to pass very small quantities of evil smelling black faeces of semifluid consistence.
- (3) On manipulation the rectum was felt to be abnormally distended.
- (4) There was no anal opening.
- (5) Further there was the presence of an external genital orifice. In other words, there was a congenital recto-vaginal fistula or cloaca (Curson, 1932).

It having been decided to destroy the animal, a post-mortem examination was held. The accompanying figure illustrates very clearly the nature of the lesion. For the size of the kitten the large intestine, and particularly the rectum, was very markedly dilated. Insertion of a probe showed that the more direct passage appeared to be towards the uterus the rectum being more of the nature of a diverticulum of the vagina. This may also be clearly seen from the figure. Strictly speaking, therefore, the external opening is embryologically a vulva.

It seems rather remarkable that the animal lived so long and also that, according to the owner, it drank a normal amount of milk.

REFERENCE.

CURSON, H. H. (1932). Anatomical Studies, No. 30: On two cases of atresia ani. This Report.



Anatomical Studies, No. 33: Micrognathy in a Lamb.

By W. J. WHEELER, Veterinary Student, Onderstepoort.

THE above condition, which is relatively uncommon, was observed in a Merino lamb forwarded to the Veterinary Research Laboratories, Onderstepoort, by Government Veterinary Officer Canham of Bloemfontein in August, 1929. Reference to the specimen (Path: No. 9551) will be seen in File 141/19, Laboratory Report 22757.



Fig. 1.

The absence of a normal mandible is well shown by Fig. 1. On dissection, it was found that not only was the tongue absent, but also that the mandible had become arrested in development. As will be seen in Fig. 2, only the vertical portion of each ramus had developed, the pars molaris being entirely absent. The two rami, which articulated normally with the squamous temporal bone, were connected at their distal ends by a strong plate of bone which seen ventrally was strongly concave from side to side.

With regard to the respiratory and digestive tracts, the position was as follows:—The nasal cavities converged posteriorly at the posterior nares and from here a narrow tube led backwards to the oesophagus and to the trachea. In other words, a diminutive

pharyngeal tube existed. The larynx, however, was abnormal only the dorsal portion of the entrance being open. At the junction of the hard and soft palate, a longitudinal slit-like opening (0.5 cm. in length), concealed by a fold of mucosa, communicated with the pharyngeal tube just referred to.



Fig. 2.

No details are available as to how long the monstrosity survived, but it is obvious that such an abnormality is incompatible with life owing to the difficulty in obtaining nourishment.

Embryologically the condition described is associated with an arrest in development of the first pair of branchial arches.

Anatomical Studies, No. 34: Faulty Jaws in Sheep.

By G. S. MARÉ, B.Sc.(Agric.), Sheep and Wool Research Officer,
Grootfontein School of Agriculture, Middelburg, C.P.

EVERY sheep farmer is familiar with the conditions known as parrot and fish mouth. In the former case the bottom jaw is short and the front teeth naturally come into contact with the palate; in the latter case the bottom jaw protrudes beyond the upper.

Both these conditions are abnormal and animals with such jaws cannot compete successfully with normal individuals, especially if the jaws are seriously out of proportion.

A concrete case of the effect of such abnormality on body growth is quoted here. A first cross Suffolk-Blackhead Persian lamb born during April, 1930, was examined and weighed at the age of 11 months. The lower jaw was 2 cm. ($\frac{3}{4}$ -inch) shorter than the upper and also slightly out of position. The teeth had actually cut into the palate, this lamb weighed 37 lb. and dressed 37.9 per cent. when slaughtered. The half-sister and brothers to this lamb, born during the same period, averaged 85.0 lb. live weight, ranging from 74 to 94 lb. and would dress from 52 to 58 per cent. (See Figure.)

This case is a clear example of how the animal with faulty jaws suffers. Normal prehension is impossible and results in starvation.

Sheep farmers are therefore well advised to watch and reject all such animals especially in the breeding flock.

Embryologically the above condition is associated with an arrest in development of the first pair of branchial arches (Wheeler, 1932).

REFERENCE.

- WHEELER, W. J. (1932). Anatomical Studies, No. 33: Micrognathia in a lamb. This Report.



Anatomical Studies, No. 35: On the Origin of an Abdominal Cyst found in a Domestic Hen.

By I. P. MARAIS, B.Sc.(Agric.), B.V.Sc., Veterinary Research
Officer, Onderstepoort.

THE presence of large pedunculated cysts attached to the cloaca is fairly commonly seen in fowls on post-mortem examination. Such cysts have been described by Curson and Martinaglia (1930) affecting the right ureter, and where the ureters were normal the cyst was said to be the persistent right Müllerian duct.



Fig. 1.

The case under review was a white Leghorn, 2nd Season hen, sent to the Veterinary Research Laboratory at Allerton, Pietermaritzburg, Natal, for post-mortem diagnosis. Photograph 1 shows the structures in position. The rectum has been drawn aside to the right.

In photograph 2 the various structures have been dissected to show the attachments. In addition to the oviduct, the rectum and the ureters, there are two other structures attached to the cloaca. On the left antero-ventral aspect there is a small tubular structure of the wall of which macroscopically in all respects resembles the wall of the oviduct, and has the appearance of an oviduct in miniature. On the right, arising from the dorso-lateral aspect of the cloaca, is a pedunculated cyst, the attachment of which appears to consist of a fold of peritoneum with blood vessels. There is also a small subsidiary cyst on one side of the stalk.

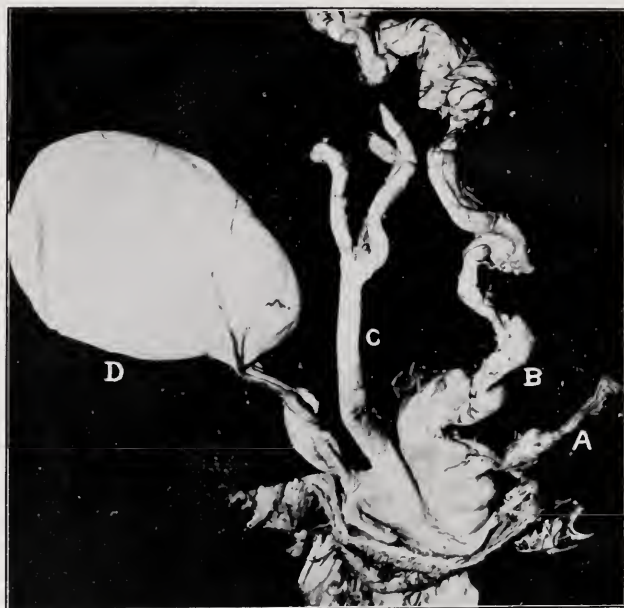


Fig. 2.

This case is of particular interest from the point of view of the origin of cloacal cysts. It shows a fully developed oviduct and what must be regarded as one partly developed, the representatives of the embryonic Müllerian ducts, and unconnected with either there is a cloacal cyst. In passing, it is of further interest to note that the fully developed oviduct is on the right side and it would appear to represent the embryonic right Müllerian duct, whereas the left normally becomes the oviduct. It is also possible that the functional oviduct represents the left Müllerian duct and that transposition has occurred during later development and in particular

owing to the presence of the cyst on the right side. Whatever the explanation may be, the material interest of this case is that both the Müllerian ducts appear to be represented and that the origin of the cyst remains to be explained. The only other explanation that can be suggested is that the cyst may represent degeneration of the Bursa of Fabricius.

REFERENCE.

- CURSON AND MARTINAGLIA (1930). Abdominal Cysts in Hens. *Vet. Rec.*, 15.2.30.

Anatomical Studies, No. 36: On Two Anomalies of the Cervix Uteri in a Merino Sheep.

By Dr. H. H. CURSON, Veterinary Research Officer, Onderstepoort.

ANOMALIES of the internal genitalia of sheep are relatively uncommon, hence a description of the above case.

Merino sheep 29237, aged approximately 12 months, was killed on 11.12.31 owing to pneumonia and the genital organs were kindly handed to me by Dr. de Kock. No history (e.g. whether a twin or not—Fraser, Roberts and Greenwood, 1928) was available. Pathological register Number 12,220.

On external examination there was a slight constriction at the anterior part of the cervix and on opening the vagina it was seen that although the orificium externum uteri (os uteri) was present, yet covering it was a strong fibrous fold arising from the vaginal floor. This so covered the os uteri that a probe inserted per vaginam failed to reach it. See figure, which represents the unopened canal.

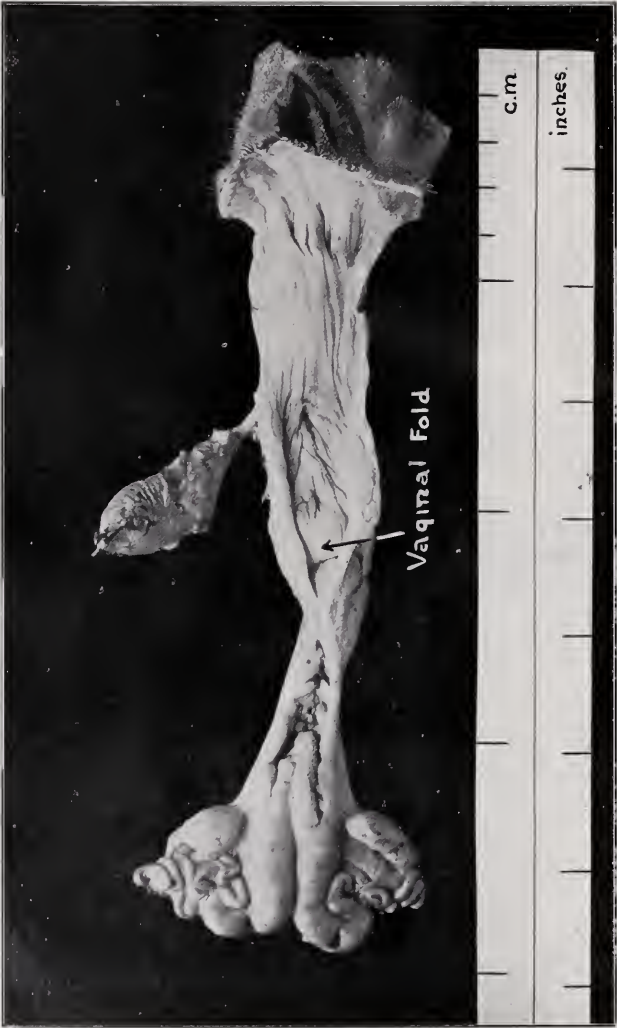
On incision of the canalis cervicalis it was further observed that the usually projecting mucous folds actually formed partitions across the lumen. Hence had penetration of the os uteri been possible, passage to the uterus would have been impossible.

Although follicles were present in both ovaries the animal had not ovulated.

The case is clearly of teratological interest, the fold described in the anterior part of the vagina being possibly misplaced, the probable normal situation being in the cervical canal. To this anomaly must be added the partitions across the lumen of the canal.

REFERENCE.

- FRASER ROBERTS, J. A., AND GREENWOOD, A. W. (1928). An Extreme Freemartin and a Free-martin-like Condition in the Sheep. *Jnl. Anat.*, Vol. 63, p. 87.



Anatomical Studies, No. 37: On a Hybrid Duiker.

By Dr. R. BIGALKE, Director, National Zoological Gardens of
South Africa, Pretoria.

FOR a number of months several Grey Duiker ewes (*Sylvicapra grimmii*) were kept in an enclosure together with a male Red Duiker (*Cephalophus natalensis*) and a female Klipspringer (*Oreotragus oreotragus*). On various occasions it was observed both by the keeper in charge and by myself that the male Red Duiker attempted to copulate with the female Grey Duikers. On the 24th September, 1931, all Grey Duiker ewes were removed from the paddock containing the male Red Duiker to another paddock containing a pair of Grey Duikers. It was in the latter paddock that the young specimen (see Figure) was found on the morning of the 7th November, 1931, in the care of its mother. The lamb had evidently been born during the night, and was almost dead when picked up; it died later in the morning, possibly as the result of exposure to rain and cold previous to having been found by the keeper.

The gestation periods of the Red and Grey Duikers do not appear to be known, but as the Grey Duiker ewe in question had been kept with the male Grey Duiker for a period of approximately six weeks only (24th September, 1931 to 7th November, 1931), when the lamb was born, it is obvious that the ewe could not have been served by the male Grey Duiker, and hence the lamb must be a hybrid between a male Red Duiker and a female Grey Duiker. This is further borne out by the lambs' colour, which is very similar to the rich rufous of the Red Duiker, and entirely different from the bluish-grey of a young Grey Duiker.

The case is of special interest, since it appears to be the first record of a hybrid between a Red and Grey Duiker. (Path. No. 12253.)

As will be observed from the accompanying figure, the under jaw was not normally developed (micrognathy).



